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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Models of additive and independent response of biological systems to mixtures of chemical agents have been modified to incorporate interactive (e.g., synergistic, or antagonistic) response. Computer programs to fit these models to experimental data have been developed. An extensive literature review was conducted to obtain data that could be used to test these models. A synoptic review of these data sets is presented. The models developed have, in general, provided an adequate degree of description of the behavior of the chemical mixtures in biological systems. The computer programs (listing provided) could be used for the analysis of data obtained in future experiments.

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Development of Novel Models for Describing Multiple Toxicity Effects

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RESTATEMENT OF OBJECTIVES

Development of appropriate standards for exposure of humans and nonhuman species to toxic materials is frequently based on an estimated risk. The estimation of risks generally relies either on epidemiologic information, or more frequently (particularly for highly toxic substances) on the extrapolation from laboratory tests on lower species. One source of considerable uncertainty in these estimates is the potential for interaction between toxic agents as they may be tested in the environment. It is difficult to test all dose combinations at which interaction may occur in the laboratory. This study developed a quantitative approach for the analysis of biological responses to exposure to mixtures of toxic materials. Therefore allowing more efficient design of laboratory testing protocols and a more precise estimation of anticipated biological effects in the ambient environment.

The work extended the isobole method of analysis of mixture toxicity and examined another method assuming independence, when no interaction occurs between components of the mixture, to analyze mixture toxicity relying quantitatively on original data (response versus concentration of chemical components in the mixture or the pure compound administered) and estimating deviations from isobole linearity quantitatively (and their concentration of effect dependence). The methods were analyzed based on binary data since there was sufficient original data to be found, however they can be adjusted for use with tertiary or higher order mixtures. The methods were tested on a variety of data of interest to the Air Force.

The additive approach using the Gibbs free energy function to express interaction was described by six different functions. The independence approach utilizes a model of statistical independence of a bivariate distribution, termed Frank's copula with a term to describe interaction.

Under the supplemental AASERT funding, the objectives were extended to include a specific test of the importance of level of effect (response) on the strength and nature of the interaction.

STATUS OF THE RESEARCH EFFORT

The effort covered by this report includes all the work completed during the length of the project. The following major activities were accomplished:

Years 0-2:

- extraction of published data into computer files
- modification of a PASCAL program for the statistical analysis of data sets using a generalized isobole approach, and testing using extracted data sets
- development of alternative solution procedures using spreadsheets

Year 3:

- development of alternative solutions using the MATLAB math program to increase computation speed and accuracy.
- development of alternative G functions which express interaction to improve models ability to describe diverse data sets.
- development of a quantitative method to describe binary dose response based on the assumption of independence and development of the MATLAB program using Frank's copula.
- comparison of the two approaches to determine differences between additivity and independence approaches.
- analyzed the influence of response on the additive models.

The specifics accomplished are noted below.

Extraction of Published Data

In the first and third years of the study, the literature was broadly surveyed for studies which potentially contained data sets in which dose response information amenable to analysis was present. During the second and third years of the study, the references were physically obtained, and data available in the published papers was compiled in a consistent form usable for analysis. All bibliographic information for each reference was placed in a data base. From this data base, another was created containing only those references actually used for statistical analysis. The data base provided in an appendix summarizes the individual papers in which usable data was found. In a number of cases, individual references contain multiple data sets. Each data set was extracted from the paper, and entered into a data file.

We classify experimental data into two basic types depending upon the nature of the response. Data in which a known, finite number of experimental subjects are assayed for an all-or-none response (e.g., tumor, death), are termed binomial (since the expected underlying error distribution is expected to be binomial). Data in which the response is graduated (e.g., fractional activity, enzyme level) are termed normal (since the expected underlying error distribution -- at least initially -- might be regarded as normal). This distinction is made since different information is required to describe and analyze the two types of studies.

For binomial data, each data set is described by a following file consisting of N+1 lines (records), where N is the number of dose combinations used. The first record is the number of dose combinations. Records 2 through N+1 are the results of each successive dose combination, containing sequentially the concentrations of the two materials in the mixture (which may include 0 for control or single component combinations), followed by the number exhibiting a positive effect, and then the total number of subjects examined.

For normal data, there are also N+1 records, with the first record being identical to the above. The successive records contain the two dose concentrations, the response value, and the standard error of the response. The last term is obtained, where possible, from the experimental data itself (many studies have experimental replicates from which this is or can be determined). In the absence of included standard error information, it is assumed that the standard error is unity (this hinders an absolute goodness of fit determination, but not the parameter estimation process per se).

The data files are coded by reference (see the reference number given in the upper left

corner of each record in the appendix), and where multiple data sets are present by the suffixes "e1", "e2", etc., denoting individual experiments within a single published paper. In other words, the data set 288e1 denotes the first data set contained in reference 288.

Model Development

Single Dose Response Models

Binary mixtures are defined as a combination of two compounds each with an individual dose response function. The equation describing the response to the two component mixture is termed the bivariate dose response function. The single dose response function of each component present in the mixture at a concentration C_i is defined as:

$$\pi_1 = f(C_1), \pi_2 = g(C_2) \qquad (1,2)$$

For simplicity the functions will be represented as f and g for $f(C_1)$ and $g(C_2)$, respectively. π represents the predicted single dose response to a compound at a concentration C_i.

For a component defined as regular each function (π_1, π_2) has the following properties:

-the dose response is continuous and bounded by 0 and 1
-the dose response is monotonically increasing

The regular response (π_i) can be linearly transformed in terms of a function $\Phi(C_i)$, where C_i is the dose of component i, as seen by:

$$\pi_i = A + B\Phi(C_i) \qquad (3)$$

A is the background response and B is the response for the maximum effect due to the compound and these are chosen such that the function Φ is monotonically increasing and bounded by 0 and 1.

These properties allow us to classify the individual dose response functions as cumulative distribution functions (Hays and Winkler 1970). The single dose response function models are shown in table 1:

Table 1 Single Dose Response Models

Model	π without Background Response	π with Background Response	Inverse Response Function π^{-1}	#
Exponential	$1 - \exp(-a_1 C)$	$1 - \exp(a_0 - a_1 C)$	$-\frac{1}{a_1}(a_0 + \ln(1-\Theta))$	(4)
Weibull	$1 - \exp(-a_1 C^{a_2})$	$1 - \exp(-a_0 - a_1 C^{a_2})$	$(\frac{-1}{a_1}(a_0 + \ln(1-\Theta)))^{(1/a_2)}$	(5)
Log-Logit	$\frac{1}{1+(\frac{1}{a_1 C^{a_2}})}$	$a_0 + \frac{1-a_0}{1+\exp(a_1 - a_2 \ln C)}$	$(\frac{\exp(a_1)*(\Theta-a_0)}{(1-\Theta)})^{(1/a_2)}$	(6)
Multi-stage	$1 - \exp(-\sum_{i=1}^{j} a_i C^i)$ j= 1,2,3...etc.	$1 - \exp(-a_0 - \sum_{i=1}^{j} a_i C^i)$ j= 1,2,3...etc.	$-a_1 + \sqrt{a_1^2 - 4a_2((a_0)+\ln(1-\Theta))}$	(7)

The a's are the dose response parameters, a0 represents the background response, j represents the total number of dose combinations and Θ is the response (also defined as π). The Weibull and logistic models were examined in this investigation since they have been shown to be effective low dose response models (Christensen and Chen 1985). The additive models proposed by Haas and Stirling (1994) use the inverse of the single dose response function with the background response, shown in column 4 above. The independence models introduce the background response in the likelihood function and, therefore, use the equations shown in column 2 of table 1.

When a response is measured on a natural scale in which response diminishes with dose, (i.e. percent activity versus control) the complement of the function can be examined, so that the cumulative distribution properties are followed. Inverse dose response functions for regular components exist such that $f^{-1}(\pi)$ defines the dose of an agent that when present alone produces a predicted response equal to π.

Additive Models

C_i is the dose of component i necessary to elicit a response (Θ) if administered alone and K_i is the dose of component i in the mixture necessary to elicit the response (Θ) then additivity can be expressed as:

$$\sum \frac{K_i}{C_i} = 1 \qquad (8)$$

when no interaction occurs between the components of the mixture (Berenbaum 1977);

(Berenbaum 1978); (Berenbaum 1985); (Berenbaum 1988); (Berenbaum 1991).

The concentrations of each single component can then be obtained by means of single dose response equations as shown in Table 1. Substituting the inverse of the single dose response function into equation (8) yields the following equation:

$$\sum \frac{K_i}{\Phi^{-1}(\Theta)} = 1 \tag{9}$$

The inverse function $\Phi^{-1}(\Theta)$ is obtained by solving the single dose response equation for this hypothetical concentration (Haas and Stirling 1994). For example, using the logit function from Table 1, the solution for each hypothetical concentration would give,

$$\Phi^{-1}(\Theta) = \left[\frac{\exp(a_1)^* (\Theta - a_0)}{(1 - \Theta)} \right] \left[\frac{1}{a_2} \right] \tag{10}$$

where a_1 and a_2 represent the single component dose response parameters, a_0 the background response and Θ the observed effect of the mixture.

When synergism occurs the basic additive equation takes the form of the following inequality, since smaller amounts of the components of the mixture are required to produce the response (Berenbaum 1977).

$$\sum \frac{K_i}{C_i} < 1 \tag{11}$$

Antagonism is shown by the opposite inequality:

$$\sum \frac{K_i}{C_i} > 1 \tag{12}$$

As an alternative hypothesis to strict isobole linearity, Haas and Stirling (1994) proposed the following:

$$\sum \frac{K_i}{C_i} = 1 + G \tag{13}$$

where G has the following property:

$$\lim_{x_i \to 1} G = 0$$

G is a function of the relative amounts of each toxin present whose fractional composition is given by:

$$x_i = \frac{K_i}{\sum_{i=1}^{n} K_i} \qquad (14)$$

The value of the interaction, G may or may not be a function of the best estimate of the predicted response ($\hat{\Theta}$) as well as the relative proportions of each component present in the mixture. Table 2 shows some alternatives that can be used to represent G. The response can then be classified as either synergistic if G is greater than zero, antagonistic of G is less than zero or if G is equal to zero then the response is termed additive (Haas and Stirling 1994).

Table 2 Equations for Excess Function G

Model Name	Expression	Equation #
Margueles 1	$A * x_1 * x_2$	(15)
Margueles 1 Plus	$(A + \hat{B}\Theta) * x_1 * x_2$	(16)
Modified Margueles 1	$\exp(A * x_1 * x_2) - 1$	(17)
Modified Margueles 1 Plus	$\exp((A + \hat{B}\Theta) * x_1 * x_2) - 1$	(18)
Margueles 2	$(A + B(x_1 - x_2)) * x_1 * x_2$	(19)
Modified Margueles 2	$\exp((A + B(x_1 - x_2) * x_1 * x_2) - 1$	(20)

Independence Models

The bivariate dose response function of the mixture is defined by the dose response functions of the two regular components. This can be termed independent action or independence when there is no change from single component response in a bivariate mixture, which means there is no interaction between the two compounds (Berenbaum 1977); (Zaider 1991). This can be shown using the concept of statistical independence with the complement of the joint response function given as the complement of the individual response functions, as follows

$$1 - H = (1 - f)(1 - g) \qquad (21)$$

or

$$\boldsymbol{H} = (1 - f)(1 - g) \qquad (22)$$

Where $\boldsymbol{H}$ is the bivariate dose response function, $\boldsymbol{H} = (1 - H)$, $x = (1 - f)$ and $y = (1 - g)$.

The bivariate dose response function is a product of the individual dose response functions, so it can be termed a bivariate cumulative distribution. Genest and Mackay (1986) highlighted a method of modelling bivariate distributions given fixed marginals using Archimedean copulas. A copula is a probability model whose function H is expressed in terms of its marginals F(x) and G(y) and the dependence function C in the form $H(x,y) = C\{F(x) + G(y)\}$ (Genest and MacKay 1986). A copula is Archimedean if it can be expressed as $a(H) = a(x) + a(y)$ (Hutchinson and Lai 1990). Frank's copula is the only Archimedean copula which includes the complete range of admissible dependence described by the Frechet bounds:

$$\boldsymbol{H} = \log_{\alpha'}\left[1 + \frac{(\alpha' x - 1)(\alpha' y - 1)}{(\alpha' - 1)}\right] \tag{23}$$

Where $\boldsymbol{H}$ is the predicted response due to both compounds with a range between 0 to 1, α' is a variable which represents the interaction between the two compounds and x and y are the inverses of the individual dose response functions. When the bivariate dose response function is in this form α' is limited between 0 and 1. However, when Frank's copula is in this form:

$$\boldsymbol{H} = -\frac{1}{\alpha}\ln\left[1 + \frac{(e^{-\alpha x} - 1)(e^{-\alpha y} - 1)}{(e^{-\alpha} - 1)}\right] \tag{24}$$

α is bounded by positive infinity and negative infinity. Figure 1 shows a plot of $\boldsymbol{H}$ versus α for $x = 0.262$ and $y = 0.475$. This form of the bivariate dose response function is termed the copula model. The interaction between the compounds as defined by Berenbaum (1977), can be determined by the sign of α. Synergism is shown when α is less than zero or when α is negative and antagonism is shown when α is greater than zero or when α is positive (Berenbaum 1977); (Berenbaum 1991). When α is equal to 0 there is no interaction between the two compounds which was earlier defined as independence. An equation for independence can be derived by solving equation (4) for the limit as α approaches 0:

$$\boldsymbol{H} = xy \tag{25}$$

$$\boldsymbol{H} = (1 - f)(1 - g) \tag{26}$$

This equation is termed the independence model, since it is a special case of the copula model to express non interaction between components in a mixture. The copula model will provide an indication of the relative interaction between the compounds.

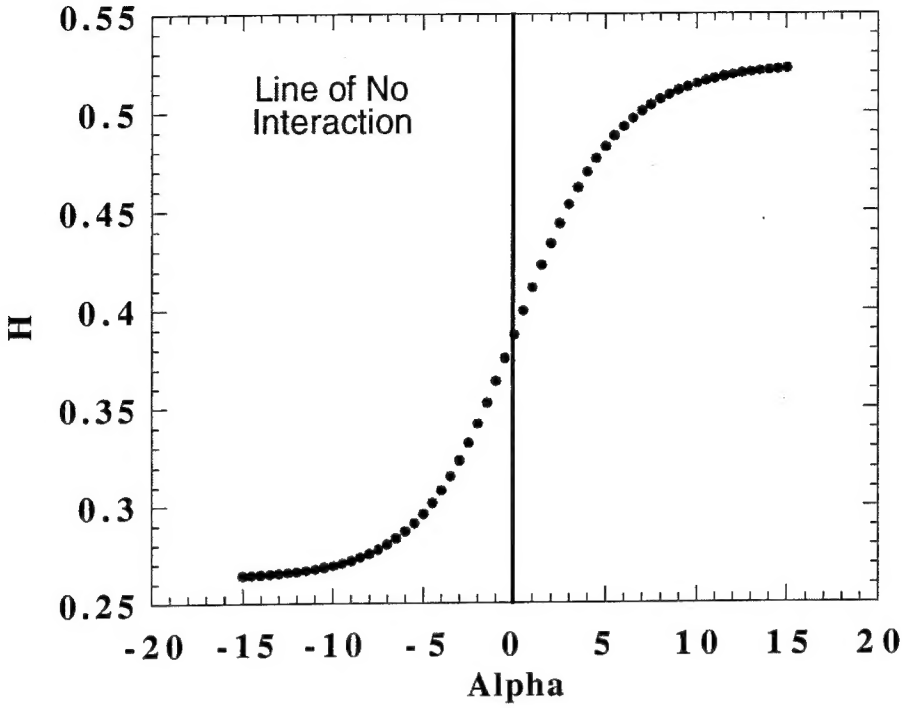

Figure 1 Plot of H versus α
x = 0.262 and y = 0.475

Method of Maximum Likelihood Estimation

The method of maximum likelihood estimation (MLE) was used to determine the best fit to the observed response. The type of data examined herein is termed binomial when the response is given as number responding versus the total sampled. Data examined in the database is termed normal when the response is given with standard deviation which was calculated for several repetitions. Given the models and parameters shown in equations (10) and (12) for the assumption of independence an estimate of the response at each dose combination can be determined from the equations:

$$\pi = a_0 + \left(1 - a_0\right)(1 - H) \qquad \text{Binomial Data} \qquad (27a)$$

$$\pi = (1 - H)a_0 \qquad \text{Normal Data} \qquad (27b)$$

$$\Theta_i = \pi$$

Θ_i is the estimate of the bivariate response for a dose concentration and a_o is equal to the background response. An estimate of the response at dose combinations for the additive models given; equation (19) and the expressions for G in Table 2 can be determined from the equation:

$$\Sigma \frac{K_i}{\Phi_i^{-1}(\Theta_i)} - 1 - G = 0 \qquad (28)$$

A solution for the response Θ_i which yields the best set of parameters, a_i's, G, and α, that provide the optimum fit to the data can be calculated by changing a function of the predicted and observed values, until a maximum or minimum Θ is achieved. The principle of the MLE is to choose the best values of Θ to maximize the following likelihood function (Chakravarti, Laha et al. 1967):

$$L\left(\Theta_1,\ldots,\Theta_n, \hat{\Theta}_1,\ldots,\hat{\Theta}_n\right) = \prod_{i=1}^{n} f\left(\Theta_i, \hat{\Theta}_i\right) \qquad (29)$$

The following likelihood equation estimates a value at each iteration of the Newton Raphson subroutine:

$$L(i) = \sum_{i=1}^{n} P(i) * \log\left(\pi(i) / \left(P(i)/T(i)\right)\right) + \left(T(i) - P(i)\right) * \log\left[\left(1 - p(i)\right) / \left(1 - \left(P(i)/T(i)\right)\right)\right] \qquad \text{Binomial Data} \qquad (30a)$$

$$L(i) = \frac{\left(\pi(i) - obs(i)\right)^2}{\sigma^2} \qquad \text{Normal Data} \qquad (30b)$$

Where P(i) is the number of responses, $\pi(i)$ is the predicted response, obs(i) is the observed response and T(i) is the sample number. The final likelihood value L(i) for the independence approach is minimized in the following equation:

$$y = -2 * L(i) \qquad (31)$$

Significance of Additive Models

The best likelihood value, the predicted response $L(\hat{\Theta})$ is evaluated in the following equation to determine statistical significance:

$$y = \frac{L(\hat{\Theta})}{L(\Theta)} \tag{32}$$

The y value is compared to the chi square distribution at (n-k) degrees of freedom, with n doses tested and k parameters to determine overall goodness of fit, a fit was accepted as significant if the p value was greater than 0.05. The p significance, the difference between the y values, was compared to a chi square distribution at 1 degree of freedom. P additions was accepted if it was less than 0.05.

Significance of Independence Models

The best likelihood value for equations 30 and 31 is compared to the chi square distribution with (n-k) degrees of freedom in equations (21) and (22) for the independence and copula models, respectively. The chi square distribution was used to determine the level of significance. The null hypothesis that the predicted values provide a significant fit to the observed values would be accepted if the likelihood value has a chi square value **greater than 0.05 with** n-k degrees of freedom.

A chi square distribution was also used to determine if the copula model provided a statistically significant better fit than the independence model. The null hypothesis that the copula model provides a better fit than the independence model if the difference between the likelihood values has a chi square value at 1 degree of freedom of **less than 0.05**.

Program Development

PASCAL Program

The initial framework used for data analysis has embodied the generalization of the Berenbaum isobole approach (Berenbaum 1976); (Berenbaum 1977); (Berenbaum 1978); (Berenbaum 1985); (Berenbaum 1988); (Berenbaum 1991) to toxicity analysis, modified by an excess function. The level of response (on a ratio scale between 0 and 1, with 0 reflecting no toxicity) to a mixture of two components (A and B) is given by:

$$\frac{d_A}{\Phi_A^{-1}(\theta)} + \frac{d_B}{\Phi_B^{-1}(\theta)} = 1 + G$$

where Φ^{-1} is the inverse dose response function (e.g., multistage, Weibull, logistic, log-probit, etc), Θ is the predicted response, d is the dose of the particular component, and G is an excess function. If G is only dependent upon the relative proportions of the two components in the mixture, it can be conveniently expressed as a function of the weight fractions of components,

denoted by x_A and x_B. Alternatively, G may also depend upon the level of response (or equivalently, on the total amount of each of the two components).

Some examples of possible functions for G, which satisfy certain necessary properties (if either component is zero, G=0) are shown in table 3:

Table 3: Functions of G

Model Name	Expression (G)
Margueles 1 (simple two suffix)	$A * x_1 * x_2$
Margueles 1 Plus	$(A + \hat{B\Theta}) * x_1 * x_2$
Modified Margueles 1 (modified two suffix)	$\exp(A * x_1 * x_2) - 1$
Modified Margueles 1 Plus (modified two suffix plus)	$\exp((A + \hat{B\Theta}) * x_1 * x_2) - 1$
Margueles 2 (three suffix)	$(A + B(x_1 - x_2)) * x_1 * x_2$
Modified Margueles 2	$\exp((A + B(x_1 - x_2) * x_1 * x_2) - 1$

NOTE: x_A and x_B represent compositional fractions of the two components

The modified model differs from the other models in allowing a more gradual departure from non-ideal behavior (G=0) at low compositional fractions. This has been found to be useful when data are obtained only at compositions which are relatively low in one component. The "plus" class of models were devised towards the end of the second year of this project to test whether any non idealities are solely a function of compositional fraction (x's), or are also a function of total mass of toxins (or, equivalently, the level of response, Θ).

The fitting program finds the best set of dose response (e.g., logistic, probit) parameters for each component in the mixture along with the best interaction parameter(s) for the chosen non ideal model. The objective function is the minimum value of -2 times the log likelihood (chosen so that a direct test of significance can be made using the chi-squared distribution). By fitting successively the ideal, margueles 1, and margueles 2 models, for example, the significance of added parameters can be determined. By the close of the second year of this project, the PASCAL program was modified to include the logistic, probit and multistage versions of the dose response models along with the Ideal, Margueles 1, and Margueles 2 non-Ideal models. The general theory underlying these approaches are described in standard references as well as in the literature on statistical aspects of risk assessment (Crump and Howe 1985); (Kendall and Stuart 1963); (Von Mises 1964).

The modified program was tested on a number of the extracted data sets to determine program robustness and performance. In this process, a number of general observations were made:

• exponential dose response relationships are more rapidly fitted, due to the simplicity of the

computation, although frequently did not yield good fits to the data (i.e., improvement using a logistic fit was often noted)

- when the underlying data was not strictly monotonic (increasing toxic effects with increasing dose), fitting tended to be poor and convergence tended to be slow. Based on this, preliminary screens for monotonicity were incorporated, and subsequent fitting has been restricted to monotonic data sets. In some cases, monotonicity could be obtained by simple transformation of either the dose scale (for example, in dietary studies, from percent protein to percent non protein) or the response scale (for normal data, from percent inhibition to percent of control activity, for example).

Computations were conducted using the THINK PASCAL compiler on Macintosh computers.

EXCEL Spreadsheets

In the course of the second year of the project, Microsoft EXCEL version 3.0 was released. This program as distributed included an optimization engine (the SOLVER add-in macro routine) which is capable of conducting both unconstrained and constrained optimization. It was felt that this might provide a more user-friendly means of conducting the data fitting that was to be undertaken in this project. Accordingly, spreadsheets were developed to conduct the fitting process in this spreadsheet environment. The particular advantages afforded by use of a spreadsheet include ease of modification to include different dose response and non ideality relationships, and ready availability of intermediate results for diagnostic purposes. To demonstrate the technique, an extensive study of one data set was conducted.

The data set used was one involving exposure of rats to two liver carcinogens - lasiocarpine (LAS) and cycad flour (CYCA) reported by NCI (Elashoff, Fears et al. 1987). These are found to fit a one and two stage multistage model, respectively. However, the goodness of fits and the specific parameters of the dose response relationship are clearly influenced by the inclusion of various forms of interaction models in the data analysis procedure. Computations have been performed using EXCEL version 3.0 (and the Solver add-in) on a Macintosh II computer. The following table reports summary statistics for example model fits:

Table 4: Comparison of Modified Margueles 1 and Modified Margueles 2 Plus

Mixture Relationship	Modified Margueles 1	Modified Margueles 2 plus
Likelihood	19.46	14.28
A	-54.74	277.2
B	N/A	-309.2
L = 2 x log likelihood (LAS)	$\Phi_{LAS} = 1 - \exp\left(0.0052 - 0.035 d_{LAS}\right)$	$\Phi_{LAS} = 1 - \exp\left(0.0054 - 0.033 d_{LAS}\right)$
L = 2 x log likelihood (CYCA)	$\Phi_{CYCA} = 1 - \exp(-0.0052 - 1.05 \times 10^{-d} d_{CYCA} - 8.40 \times 10^{-9} d^2_{CYCA})$	$\Phi_{CYCA} = 1 - \exp(0.0054 - 1.22 \times 10^{-d} d_{CYCA} - 4.2 \times 10^{-9} d^2_{CYCA})$

On the basis of this analysis it is concluded that (1) the non-ideal interactions are highly significant from a statistical point of view as reflected in the significance of the reduction in the likelihood statistic from the ideal model (not shown, but L=30.5 for the ideal model fit) and (2) the modified two suffix plus model is necessary to provide an acceptable degree of fit (for 10 degrees of freedom, this is at about the 5 % significance level). It was found that the spreadsheet, although perhaps slower in terms of execution time, could produce results which are significant, and which are easier to understand. Accordingly, by the end of year 2 of this project, a decision was made to transfer subsequent work to a spreadsheet environment.

The practical significance of these results are illustrated by Figure 2. In this plot, the dose-response functions described by the three mixture models are shown for a hypothetical mixture consisting of 1 % LAS and 99 % CYCA. It is clear that there is over an order of magnitude difference in the estimated amount of the mixture which assures an incremental risk (above background) in the $< 10^{-2}$ range. Thus, the quantitative incorporation of mixture effects would have a significant effect on setting acceptable exposure limits to such materials. Furthermore, this figure shows that the incorporation of a dependency of the excess function (G) on level of response (Θ) has a major effect on the estimated "safe" dosage. Therefore incorporation of these effects, when they are of statistical significance, has major practical consequences.

These findings stand in sharp contrast to the frequently stated assumptions regarding mixture toxicity. Essentially, many individuals, as well as current regulatory practice, believe that although mixture interactive effects may be significant at high doses, they may be ignored at low doses and risks in favor of assumptions of additive toxicity (Krewski 1989); (US EPA 1986).

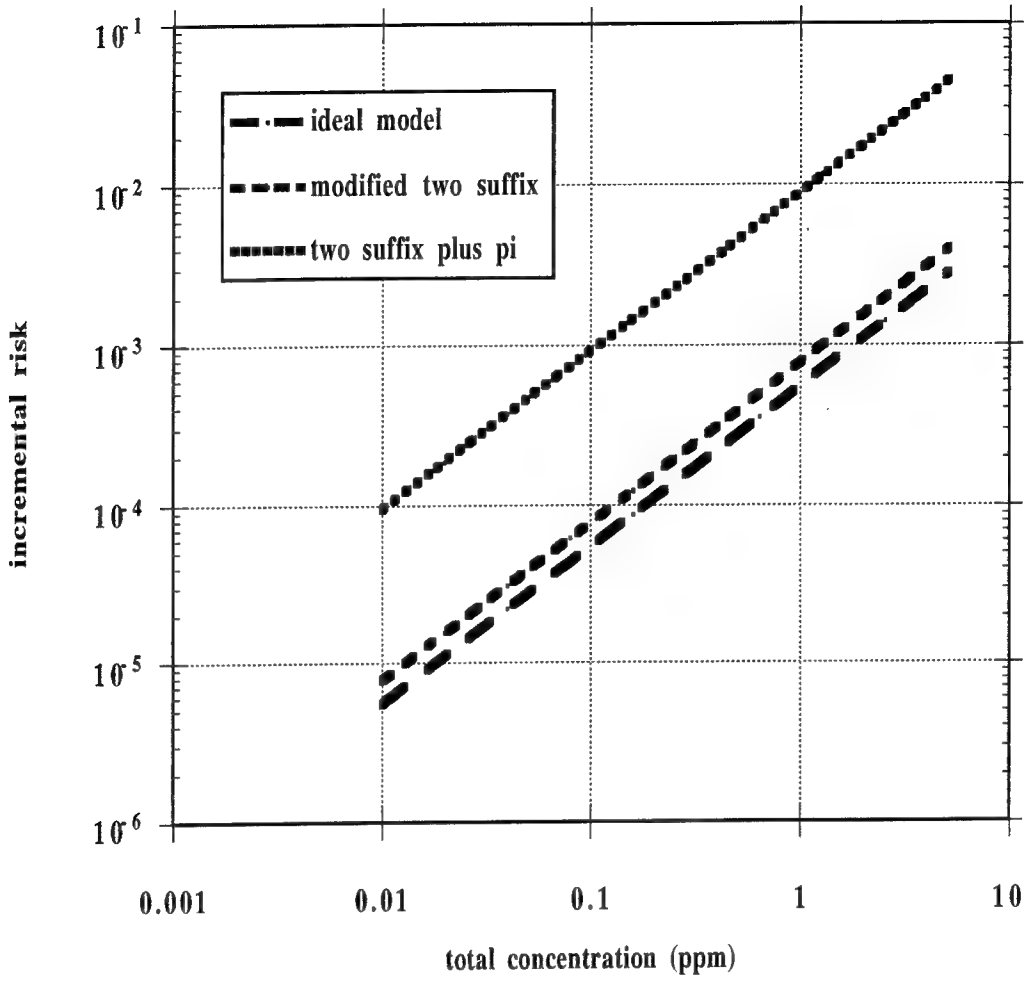

Figure 2: Predicted Response to a Mixture with 0.01 Weight Fraction of LAS Using Different Mixture Models.

MATLAB Program

The release of the MATLAB program allowed further evolution of the computation process. Much like the EXCEL program, MATLAB allowed diagnostic analysis of the results, but it also allowed further constraints such as a maximum number of iterations and it allow variability of the final constraints for data sets which were difficult to fit. This was especially important when the fitting took too much time. The MATLAB program was also faster than the EXCEL program and provided smaller likelihood values for some data sets.

There were two programs written for the additive and independence approaches, one for binomial data and the other for normal data sets. The program utilized equation 13 for the additive models and equations 24 and 26 for the independence models. The MATLAB program incorporated diversity by allowing the individual dose response functions for the two components to be changed to either weibull or logistic.

The MATLAB program was started by first guessing a set of initial parameters, k's then starting the program. The initial values were used in the program to make an initial estimate of the likelihood function, this value was compared to the constraints, evaluated and another guess made using a Newton Raphson iteration process. An EXCEL spreadsheet was used to determine initial guesses for those data sets that were difficult to fit.

The MATLAB subroutine 'fminu' was employed to utilize the Newton-Raphson iteration. The subroutine uses a Nelder-Meade simplex search algorithm. The Nelder-Meade method is a direct search method that does not use gradients or other derivative information. Given, n is equal to the length of x, a simplex in n-dimensional space is characterized by the n+1 vectors, also the simplexies vertices. A simplex is a triangle in 2-D space and a pyramid in 3-D space. At each step of the iteration a new value is generated near the simplex vertices. The new points are compared to the values of the current simplex and the new value is accepted if the new value will make the simplex closer to the specified constraints (MathWorks 1992) The complete MATLAB program used is shown in appendix B.

Model Evaluation

The models were evaluated using data sets from the database which met the constraints of the model. The data sets are referenced by author, date and experiment number.

Additive Models

Air Force Toxins

The first analysis involved experimental data from Bulusu, S. and Chakravarthy, I (1988). The experiment studied drug metabolizing enzymes in rats treated with parathion, malathion and phosalone under various conditions of protein energy malnutrition.

The experiments were performed upon adult male albino Charles Foster strain rats weighing 100-120 grams. The rats were divided into three dietary groups of 90 rats each. Isoenergetic diets consisting of 16%, 6% and 3% casein protein respectively were fed to the rats for three consecutive weeks. Food and water were given **ad libitum** and all animals were weighed weekly.

Parathion, malathion and phosalone on daily administration for 21 consecutive days caused significant reductions in the hepatic aniline hydroxylase activity in both the normal and the protein deprived rats. Data analysis for the above data was carried out using the logistic equation to represent the components of the mixture. The response measured was the aniline hydroxylase activity. In order to satisfy the requirements of data analysis, the data was changed from the % protein to % non-protein in each case.

The data is listed in table 5 and the model parameters are listed in table 6. Additive models for the excess function G passed the overall goodness of fit test. The Modified Margueles 1 model provided the most statistically significant fit to the data with a P-additions of 0.0039. The G-value

for the above model calculated from model parameters indicates that the combination of protein and malathion is antagonistic.

Table 5: Data for Bulusu and Chakravarty (1988), #54e2
Malathion and Percent Protein
Converted to Percent Non Protein
Testing Aniline Hydroxylase Activity

Protein (%)	Non Protein (%)	Malathion (ug/k)	Aniline Hydroxylase Activity	Standard Deviation
16	84	0	1.88	0.06
16	84	50	1.71	0.12
16	84	100	1.53	0.13
16	84	150	1.13	0.09
16	84	200	1.12	0.14
6	94	0	1.43	0.09
6	94	50	1.04	0.15
6	94	100	0.97	0.10
6	94	150	0.95	0.08
6	94	200	0.75	0.06
3	97	0	1.18	0.07
3	97	50	1.01	0.08
3	97	100	0.91	0.06
3	97	150	0.80	0.11
3	97	200	0.63	0.11

Table 6. Model Parameters for Bulusu, S. and Chakravarthy, I (1988), #54e2
Malathion and Percent Protein
Logistic Equation

Parameters	Ideal Model	Margueles 1	Margueles 1 Plus	Modified Margueles 1	Modified Margueles 1 Plus	Margueles 2	Modified Margueles 2
a0	1.4778e7	540.93	2984.6	1.1250e6	2.0130e6	262.27	316.74
a1-cmpd 1	3.0480	8831.9	2.2302	4.7116	2.8689	2.5707e4	2.0237e4
a2-cmpd 1	3.8355	3.3284	3.4036	3.3522	3.3710	3.4029	3.3918
a1-cmpd 2	2.0662e-6	8176.2	8.5096e-5	7.0610e-2	19.868	5.5474	5.6041
a2-cmpd 2	0.4396	1.8548	0.6789	1.3708	2.1201	0.94098	9.6414
A	N/A	-0.2681	-0.3220	-0.2686	-0.1721	-0.1318	-0.1451
B	N/A	N/A	-7.944e-2	N/A	-0.9536	-0.3320	-0.3249
2 Ln L	17.654	9.4881	9.9554	9.3458	9.2813	8.2593	8.2111
P (overall)	6.1100e-2	0.3935	0.2682	0.4060	0.3191	0.4086	0.4131
P (addns)	N/A	4.300e-2	N/A	0.0040	0.6493	0.2677	0.2585

This second experiment presented herein by Sun and Johnson (1960) involved the analysis of the joint action of insecticides against houseflies. The experiment under analysis used pure aldrin and dieldrin. The percent concentration of each toxicant was expressed as grams of active ingredient per 100 ml of refined kerosene (No. 10) solution. The house fly, *Musca domestica L.*, National Association of Insecticide and Disinfectant Manufacturers (NAIDM) 1948 strain, was reared at 80 ± 2^0 F and 40 % to 50 % relative humidity. and tested under the same conditions by the horizontal spray tunnel method. Four day old flies were thoroughly mixed and properly sampled before counting 100 individuals into each cage. Four concentrations of each toxicant and mixture were tested. The whole series was tested on the same day and on different days. The data for this experiment is shown in table 7.

Table 7: Data for Sun, Y.P. and Johnson E.R. (1960), #283e1
Aldrin and Dieldrin
House Fly Mortality
Converted to Percent Non Mortality

Aldrin	Dieldrin	Mortality (%)	Non Mortality (%)	Standard Deviation
0.0075	0.00	35.3	64.70	2.08
0.01	0.00	55.3	44.70	9.86
0.015	0.00	64.70	35.30	3.79
0.02	0.00	88.70	11.30	3.79
0.00	0.0035	38.00	62.00	8.89
0.00	0.005	53.70	46.30	10.97
0.00	0.0075	72.30	27.70	3.21
0.00	0.01	83.00	17.00	3.61
0.00333	0.00167	37.30	62.70	4.20
0.005	0.0025	53.00	47.00	4.00
0.0075	0.00375	73.00	27.00	11.10
0.01	0.005	86.00	14.00	4.90

The results of the analysis for the above data are shown in Table 8. All the proposed models yield good fits and pass the overall goodness of fit test. However, since the P-additions are greater than 0.05 the ideal model is accepted as the best fit, indicating no interaction

Table 8. Additive Model Parameters for Sun Yun Pei and Johnson, E.R. (1960), #283e1
Logistic Model.
House Fly Mortality
Aldrin and Dieldrin
Converted to Percent Non Mortality

Parameters	Ideal Model	Margueles 1	Margueles 1 Plus	Modified Margueles 1	Modified Margueles 1 Plus	Margueles 2	Modified Margueles 2
a0	67.953	68.018	67.953	68.017	67.591	67.953	67.964
a1-cmpd 1	8.8276e-9	9.5292e-9	8.8284e-6	9.5198e-9	4.8367e-9	8.8302e-9	9.0157e-9
a2-cmpd 1	4.3873	4.3755	4.3873	4.3757	4.5431	4.3872	4.3894
a1-cmpd 2	1.9783e-6	8.6170e-7	1.9785e-6	8.6080e-7	4.3113e-7	1.9780e-6	8.7681e-7
a2-cmpd 2	2.6040	2.7840	2.6040	2.7842	2.9293	2.6040	2.7807
A	N/A		-0.2659	-0.9728	-7.602e-2	-0.6389	5.7796
B	N/A	N/A	-0.4335	N/A	-6.5800e-3		
2 Ln L	4.1649	3.5545	4.1649	3.5545	3.4852	4.1649	3.5389
P (overall)	0.7606	0.7367	0.5259	0.7367	0.6256	0.5259	0.6175
P (addns)	N/A	0.4346	N/A	0.4346	0.7923	N/A	0.9007

The next work presented compared the chronic and acute responses to chemical mixtures. The purpose of this experiment (Hermanutz, Eaton et al. 1985) is [1] to determine possible differences between the toxicity of individual concentrations and mixtures of them for two different pesticide types the organophosphate malathion and the chlorinated cyclodiene endrin and [2] to determine differences in the chronic joint toxicity and acute joint toxicity for a pesticide mixture on flagfish mortality.

Two 2 liter proportional diluters were synchronized to deliver control water and 3 endrin concentrations and control water and 3 malathion concentrations. Individual concentration selections were based on data from an earlier experiment by Hermanutz, which indicated that one or more of the three concentrations would produce between 25 and 75 % reductions in survival or growth or both.

Two experiments from this study were are presented herein. The first experiment involved the mortality of 60 flagfish on their 30th exposure day in the chronic exposure and the data is shown in table 9, #121e11. The second experiment involved the mortality of 80 juvenile flagfish during acute exposure after 48 hour and the data is shown in table 12, #121e13. The additive model parameters are shown in tables 10 and 11, for the logistic and Weibull equations respectively. The model parameters from the acute mortality experiment are shown in tables 13 and 14 for the logistic and Weibull equations.

The logistic equation describes the data well with the Modified Margueles 1 plus model providing the best statistical fit to the data, with a p value of 0.766 and a p additions of 0.002. The fit of the Modified Margueles 1 plus model indicates interaction between the compounds and a

positive G value indicates synergism.

Table 9: Data for Hermanutz, R.O. et al. (1985), #121e11
30 Day Mortality of Adult Flagfish
Endrin and Malathion

Endrin (ug/L)	Malathion (ug/L)	Number Dead	Flagfish Sampled
0.21	0.00	9	60
0.29	0.00	15	60
0.39	0.00	46	60
0.00	13.8	5	60
0.00	18.5	6	60
0.00	23.1	8	60
0.20	15.3	11	60
0.21	19.7	22	60
0.22	23.3	26	60
0.28	15.6	18	60
0.28	19.8	36	60
0.42	13.7	57	60

The results of the Weibull equation to describe the individual dose response are shown in table 11. All the additive models, with the exception of the ideal model significantly fit the data. The Modified Margueles 1 plus model provided the best overall fit to the data.

Table 10: Additive Model Parameters for Hermanutz et al. (1985), #121e11
Logistic Model
30 Day Mortality of Adult Flagfish
Endrin and Malathion

Parameters	Ideal	Margueles 1	Margueles 1 Plus	Modified Margueles 1	Modified Margueles 1 plus	Margueles 2	Modified Margueles 2
a0	4.4578e-15	0.1257	9.9654e-2	0.1253	9.8970e-2	0.1080	0.1089
a1 Cmpd 1	-7.1405	-12.347	-8.9138	-12.326	-8.8667	-9.6798	-9.7721
a2 Cmpd 1	6.2538	11.931	8.3911	11.914	8.3426	9.1606	9.2517
a1 Cmpd 2	5.2603	25.795	14.862	24.713	14.325	25.569	25.250
a2 Cmpd 2	1.0494	6.1438	3.6995	5.8571	3.5358	6.9848	6.8346
A	N/A	4.8366	27.015	4.3002	23.564	-570.21	-460.38
B	N/A	N/A	-2.9165	N/A	-25.854	-611.33	-494.05
Likelihood	26.971	11.774	2.7380	11.907	2.5688	5.4872	5.4956
p - value	3.3722e-4	6.7211e-2	0.7403	6.4080e-2	0.7661	0.3593	0.3584
p - add	N/A	9.6815e-5	2.6475e-3	1.0389e-4	2.4136e-3	1.2166e-2	1.2223e-2

Table 11: Additive Model Parameters for Hermanutz et al. (1985), #121e11
Weibull Model
30 Day Mortality of Adult Flagfish
Endrin and Malathion

Parameters	Ideal	Margueles 1	Margueles 1 Plus	Modified Margueles 1	Modified Margueles 1 plus	Margueles 2	Modified Margueles 2
a0	9.3010e-2	0.1198	9.4879e-2	0.1196	9.4395e-2	0.1047	0.1058
a1 Cmpd 1	427.38	3.8359	466.48	3936.6	451.52	858.11	904.34
a2 Cmpd 1	6.2079	8.4347	6.2139	8.4642	6.1794	6.8510	6.9061
a1 Cmpd 2	4.4411e-5	2.6998e-12	2.6161e-7	8.1244e-12	3.6780e-7	1.3729e-10	1.2581e-10
a2 Cmpd 2	2.0995	6.4087	3.7560	6.1117	3.6483	5.9729	5.9434
A	N/A	6.3734	23.739	5.6879	20.687	-348.42	-282.97
B	N/A	N/A	-20.131	N/A	-17.439	-378.68	-308.07
Likelihood	12.532	7.3632	2.5679	7.5372	2.4068	4.4984	4.4745
p - value	8.43e-2	0.2887	0.7662	0.2740	0.7905	0.4801	0.4833
p - add	N/A	2.2995e-2	2.8536e-2	2.5423e-2	2.5993e-2	9.0537e-2	8.9200e-2

Table 12: Data for Hermanutz, R.O. (1985), #121e13
48 Hour Mortality of Juvenile Flagfish
Endrin and Malathion

Endrin (ug/L)	Malathion (ug/L)	Number Dead	Flagfish Sampled
0.59	0.00	1	80
0.76	0.00	0	80
0.86	0.00	1	80
0.00	265	26	80
0.00	331	45	80
0.00	419	60	80
0.58	272	33	80
0.70	278	29	80
0.96	259	36	80
0.58	359	51	80
0.72	360	53	80
0.54	455	72	80

The model parameters for the acute mortality experiment are shown in tables 13 and 14 for the logistic and Weibull models, respectively. The Ideal model provided the best overall fit to the data, since non of the higher order models provided significant improvements in the fit. The logistic equation provided a better fit than the Weibull equation. The Ideal model indicates that the compounds do not interact at these concentrations, perhaps due to the skewed concentration ratios. The malathion concentration is about 100 times greater than the endrin concentration, effectively eliminating the response due to endrin.

Table 13: Additive Model Parameters for Hermanutz et al. (1985), #121e13
Logistic Model
48 Hour Mortality of Juvenile Flagfish
Endrin and Malathion

Parameters	Ideal	Margueles 1	Margueles 1 Plus	Modified Margueles 1	Modified Margueles 1 plus	Margueles 2	Modified Margueles 2
a0	6.2108e-3	5.3433e-3	8.6088e-3	5.6606e-3	1.0058e-2	6.7679e-3	6.4751e-3
a1 Cmpd 1	4.8452	13.312	14.144	9.5021	12.391	5.8295	5.5652
a2 Cmpd 1	4.3110	2.5593	0.1901	2.6067	2.6986	1.9502	2.2395
a1 Cmpd 2	18.820	19.317	20.389	19.177	19.499	19.499	19.877
a2 Cmpd 2	3.3488	3.4358	3.6234	3.4103	3.4664	3.4664	3.5342
A	N/A	-73.951	-214.88	-78.750	-122.19	-29.225	-3852.1
B	N/A	N/A	202.21	N/A	58.996	36.954	-3811.3
Likelihood	8.9093	8.6407	7.1143	8.6652	8.0464	8.2532	8.1819
p - value	0.2592	0.1948	0.2122	0.1933	0.1537	0.1428	0.1465
p - add	N/A	0.6043	0.2166	0.6212	0.4407	0.5336	0.4982

Table 14: Additive Model Parameters for Hermanutz et al. (1985), #121e13
Weibull Model
48 Hour Mortality of Juvenile Flagfish
Endrin and Malathion

Parameters	Ideal	Margueles 1	Margueles 1 Plus	Modified Margueles 1	Modified Margueles 1 plus	Margueles 2	Modified Margueles 2
a0	6.0374e-3	8.2026e-3	4.3204e-10	7.4056e-3	8.3851e-3	1.7240e-61	6.3458e-3
a1 Cmpd 1	7.6297e-3	1.8073e-4	8.7312e-3	1.4761e-3	3.2195e-5	8.3682e-3	2.1398e-3
a2 Cmpd 1	3.8948	1.3005	1.0489e-7	1.4830	1.0269	1.4619e-7	2.0227e-2
a1 Cmpd 2	2.6183e-5	1.1252e-5	4.4806e-6	1.3322e-5	7.8887e-7	1.2338e-5	8.5967e-6
a2 Cmpd 2	1.8219	1.9663	2.1275	1.9368	2.4246	1.9519	2.0152
A	N/A	-77.878	-190.44	-85.3789	-388.13	-38.789	-5639.8
B	N/A	N/A	163.76	N/A	432.22	39.622	-5586.8
Likelihood	9.5105	8.1410	6.7079	8.3604	6.1857	8.1394	8.0102
p - value	0.2180	0.2279	0.2433	0.2129	0.2886	0.1487	0.1557
p - add	N/A	0.2419	0.2313	0.2835	0.1620	0.9675	0.7176

The experiment conducted by Weber et al., (1985) involved the examination of the teratogenic potency of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) both singly and in combination in C57BL/6N mice. TCDD and TCDF are polychlorinated aromatic hydrocarbons which are unwanted byproducts of useful industrial chemicals. They pose a significant threat to humans due to their chemical and biological stability which causes them to accumulate in the food chain.

The TCDD and TCDF were dissolved in reagent grade acetone mixed with commercial corn oil for treatment purposes. The acetone was then removed by evaporation under vacuum. The mice being used in this study were female C57BL/6N mice weighing 17-20 grams. They were mated overnight with proven C57BL/6N males and were checked the following morning for the presence of the vaginal plug. On gestation days 6 and 10, the females were weighed to confirm pregnancy and were randomly assigned to various treatment groups. The female mice were then killed by decapitation on gestation day 18. The fetuses were weighed individually and fixed in Bouin's solution for 4-5 days. The fetuses were then examined for the presence or absence of cleft palates by a cut between the upper and the lower jaws.

The experimental data is shown in Table 15. The statistical analysis using the above data was performed using both the logistic and the Weibull models to represent the components of the mixture respectively. Results of the statistical analysis is shown in Table 16 (Logistic) and Table 17 (Weibull).

Table 15: Data for Weber et al. (1985), #293
TCDD and TCDF
Cleft Palate Formation in Mice Litters

TCDD (ug/kg)	TCDF (ug/kg)	Affected Litters	Sample Litters
12.00	0.00	4	10
17.00	0.00	9	11
22.00	0.00	10	10
0.00	300.00	3	11
0.00	600.00	9	10
0.00	900.00	7	7
12.00	300.00	10	10
12.00	600.00	11	11

All the additive models pass the overall goodness of fit test when the logistic equation is used to describe the individual dose response, see table 16. However, the higher order models do not provide a significant improvement in fit over the Ideal model, indicating no interaction between the compounds.

The model parameters from the statistical fitting to the data using the Weibull equation are listed in table 17. The Ideal model provides the best overall fit to the data with a p value of 0.908. However the other models were not tested since the likelihood ratio is less than 1 and an

improvement in the fit is not possible. This indicates that the compounds are not interacting at the doses tested. So, it can be concluded that there is no interaction between TCDD and TCDF, since the Ideal model describes additivity.

Table 16: Additive Model Parameters for Weber et al. (1985), #293e1
Logistic Model
Cleft Palate Formation in Mice Litters
TCDD and TCDF

Parameters	Ideal	Margueles 1	Margueles 1 Plus	Modified Margueles 1	Modified Margueles 1 plus	Margueles 2	Modified Margueles 2
a0	7.7676e-13	0.3173	0.2877	0.2922	0.3332	0.3363	0.3366
a1 Cmpd 1	18.285	35.072	25.661	27.461	106.06	73.694	62.233
a2 Cmpd 1	7.1494	12.775	9.5052	10.136	37.783	26.356	22.312
a1 Cmpd 2	31.102	177.46	67.433	96.251	166.46	165.87	168.88
a2 Cmpd 2	5.2736	28.017	10.826	15.329	26.293	26.199	26.670
A	N/A	-15.042	-14.195	-45.654	-7.1435	-5.1960	-4.4698
B	N/A	N/A	-11.619	N/A	-6.4821	-4.4359	-3.3282
Likelihood	1.7640	0.4342	0.4679	0.4398	0.3827	0.3968	0.4072
p - value	0.6228	0.8049	0.4821	0.8025	0.5361	0.5287	0.5233
p - add	N/A	0.2488	N/A	0.2498	0.8205	0.8467	0.8696

Table 17: Additive Model Parameters for Weber et al. (1985), #293e1
Weibull Model
Cleft Palate Formation in Mice Litters
TCDD and TCDF

Parameters	Ideal	Margueles 1	Margueles 1 Plus	Modified Margueles 1	Modified Margueles 1 plus	Margueles 2	Modified Margueles 2
a0	2.8900e-5	Not run	since the	Ideal	model fit	can not be	improved
a1 Cmpd 1	1.8823e-5						
a2 Cmpd 1	4.0713						
a1 Cmpd 2	1.1568e-8						
a2 Cmpd 2	2.9981						
A	N/A						
B	N/A						
Likelihood	0.5507						
p - value	0.9076						
p - add	N/A						

Non Air Force Toxins

The thrombolytic agents recombinant human tissue type plasminogen activator (rt-PA) and recombinant human single chain urokinase-type plasminogen activator (rscu-PA) both have a short life in humans. This is mainly due to rapid hepatic clearance. Their use as a therapeutic agent therefore requires that they be ingested in relatively large amounts.

The use of combinations of reduced doses of the two agents has been studied in an effort to increase the potency rates at the same time minimizing the side effects. In the present study Lu, H.R. et al., 1991 studied the thrombolysis with single and combined intravenous infusion or bolus injection of rt-PA-ΔFE/scu-PA-e, rt-PA, and rscu-PA in a hamster pulmonary embolism model.

The analysis presented herein involved data from the effect of rt-PA and rscu-PA when administered as a bolus injection was studied, the data is shown in table 18. The response was transformed from % clot lysis to % non clot lysis. The experimental data was analyzed using the logistic equation to represent each of the thrombolytic agents. Table 19 shows the results of the statistical analysis using the logistic equation to describe the individual dose response.

Table 18: Data for Lu, H. R. et al. (1991), #294e7
rscu-PA and rt-PA-ΔFE/scu-PA-e by Intravenous Infusion
Converted to Percent Non Clot Lysis

rscu-PA	rt-PA-ΔFE/scu-PA-e	Percent Clot Lysis	Percent Non clot Lysis	Standard Deviation
0.25	0.00	33	67	2
0.50	0.00	63	37	3
1.00	0.00	83	17	3
2.00	0.00	92	8	3
0.00	0.016	28	72	3
0.00	0.032	58	42	6
0.00	0.064	93	7	1
0.125	0.008	31	69	3
0.25	0.016	47	53	6
0.50	0.032	84	16	1

Table 19: Additive Model Parameters for Lu, H.R. et al. (1991),#294e7
Logistic Model
rscu-PA and rt-PA-ΔFE/scu-PA-e by Intravenous Infusion
Converted to Percent Non Clot Lysis

Parameters	Ideal Model	Margueles 1	Margueles 1 Plus	Modified Margueles 1	Modified Margueles 1 Plus	Margueles 2	Modified Margueles 2
a0	80.848	80.000	80.000	80.000	80.000	80.000	80.000
a1-cmpd 1	0.2149	0.2123	0.2183	0.2124	0.2185	0.2174	0.2174
a2-cmpd 1	1.3413	1.4318	1.4066	1.4317	1.4060	1.4104	1.4104
a1-cmpd 2	0.5629	0.6434	0.6703	0.6439	0.6708	0.6667	0.6667
a2-cmpd 2	1.6010	1.7500	1.6697	1.7492	1.6680	1.6803	1.6803
A	N/A	-3.7099	-5.9730	-4.6390	-8.2941	181.347	287.29
B	N/A	N/A	5.1322e-2	N/A	8.1508e-2	238.46	376.25
2 Ln L	24.901	4.7433	2.9975	4.7030	2.9776	3.2458	3.2458
P (overall)	0.0016	0.6913	0.8092	0.6961	0.8116	0.7774	0.7774
P (addns)	N/A	7.1309e-6	N/A	6.9800e-6	0.1839	0.2210	0.2210

Plummer and Short (1991) tested combinations of Thiopentone and Midazolam. In the experiment presented herein, the effect of thiopentone and midazolam administered both individually and together to people was studied. The proportion of people falling asleep was measured following exposure to the drugs. The data is shown in table 20.

Table 21 shows the model parameters using the logistic equation to describe the data. None of the models significantly fit the data. The statistical analysis was repeated using the Weibull equation as the single component dose response equation, see table 22.

All the additive models provide significant fits to the data. In addition, the Margueles 1 and the Modified Margueles 1 models provide significant improvement in fits over the Ideal model indicating antagonistic interaction.

Table 20: Data for Plummer and Short (1990), #299e1
Thiopentone and Midazolam
Recorded the Number Asleep

Thiopentone (mg/kg)	Midazolam (mg/kg)	Subjects Asleep	Total Subjects
2.5	0.00	5	20
3.0	0.00	9	20
3.5	0.00	15	20
4.0	0.00	18	20
0.00	0.100	4	20
0.00	0.125	7	20
0.00	0.150	8	20
0.00	0.175	10	20
0.00	0.200	14	20
0.7	0.04	3	20
1.1	0.06	9	20
1.5	0.08	15	20
1.9	0.10	19	20
2.3	0.12	19	20

Table 21: Model Parameters for Plummer, J.L. and Short, G.T., (1990), #299e1
Logistic equation.
Recorded the Number Asleep
Thiopentone and Midazolam

Parameters	Ideal Model	Margueles 1	Margueles 1 Plus	Modified Margueles 1	Modified Margueles 1 Plus	Margueles 2	Modified Margueles 2
a0	0.3309	0.2106	5.5072e-06	0.2056	0.1952	1.7743e-01	0.1676
a1-cmpd 1	13.024	11.681	6.9660	11.433	9.5148	9.0333	8.3757
a2-cmpd 1	10.811	9.9109	6.3825	9.7148	8.1443	7.7768	7.2427
a1-cmpd 2	1.5877e-6	3.9903e-7	2.8998e-6	4.7826e-8	4.2108e-6	3.4037e-22	4.4292e-22
a2-cmpd 2	0.5678	0.7218	0.2831	0.7041	6.7321e-6	0.6022	0.5649
A	N/A	-10.7701	-9.1853	-15.259	-8.8303	664.04	1865.3
B	N/A	N/A	-3.4493	N/A	-9.0553	-750.89	-2092.6
2 Ln L	56.413	18.1099	18.1753	17.886	17.128	16.076	15.547
P (overall)	6.5438e-9	0.02042	0.0112	0.0221	0.01658	2.443e-2	0.0296
P (addns)	N/A	6.0556e-10	N/A	5.4002e-10	0.3218	0.1538	0.1094

Table 22: Additive Model Parameters for Plummer, J.L. and Short, G.T.(1990),#299e1
Weibull Model
Recorded the Number Asleep
Thiopentone and Midazolam

Parameters	Ideal Model	Margueles 1	Margueles 1 Plus	Modified Margueles 1	Modified Margueles 1 Plus	Margueles 2	Modified Margueles 2
a0	0.3331	0.2106	5.5072e-6	9.1725e-10	0.1952	0.17743	8.269e-26
a1-cmpd 1	13.024	11.681	6.9660	6.0169e-3	9.5148	9.0333	5.4729e-3
a2-cmpd 1	10.811	9.9109	6.3825	4.2756	8.1443	7.7768	4.3551
a1-cmpd 2	1.5877e-6	3.9903e-7	2.8998e-6	27.092	4.2108e-6	3.4037e-22	34.391
a2-cmpd 2	0.5678	0.7218	0.2831	2.0338	0.6732	0.6021	2.1639
A	N/A	-10.770	-9.1853	-4.9147	-8.8302	664.04	-176.33
B	N/A	N/A	-3.4493	N/A	-9.0553	-750.89	191.01
2 Ln L	13.408	2.5575	2.4042	2.5617	2.3789	2.5001	2.4871
P (overall)	0.1450	0.9589	0.9341	0.9587	0.9359	0.9270	0.9280
P (addns)	N/A	0.0009	N/A	0.0009	0.6725	0.8107	0.7907

Influence of Response on the Additive Models

Based on the above results, we can conclude that the proposed models for the excess function G provide significant fits when coupled with either the logistic or the Weibull equation to represent the components of the binary mixture. Table 23 shows a summary of the data sets presented herein, where the plus models provided statistically significant improvement in fit over the Ideal model.

Table 23: Summary of data sets

Experiment #	Equation Used	Model with best fit
121e11	logistic	Modified Margueles 1 Plus
121e11	Weibull	Modified Margueles 1 Plus
121e13	Weibull	Modified Margueles 1 Plus
294e13	logistic	Modified Margueles 1 Plus

In addition to the experiments displayed in table 23, there were instances where the margueles 1 plus model passed the overall goodness of fit test at the 5 % level of significance but did not pass the test for improvement of fit as a result of the added parameter over the margueles 1 model because of the low likelihood ratio value obtained in both the cases as a result of which there is not going to be any improvement in fit below a certain value of likelihood.

The Margueles 1 Plus and the Modified Margueles 1 Plus models both incorporate the response as one of the parameters on which the excess function is dependent upon. Considering the results provided by the plus and the modified plus models we can say that the response influences the ability of the additive models to provide better fits when compared to the other models since the models which incorporate response yield better fits than those models which do not have the response as one of the dependent parameters.

Summary of Additive Models

A total of 37 data sets, including those with outliers removed were analyzed over the course of the study. The additive models provided a statistically significant fit for nineteen data sets. From the data sets that were fit, the ideal model fit eight of them, this indicates non-interaction and thus interaction for the other eleven. The plus models provided a statistically significant fit for six data sets from which we can conclude that interaction has an effect on the response.

Results of the Independence Models

Generally, the independence models did not provide statistically significant fits to the data. However, the MATLAB program did run faster than the additive model program. The independence models did not predict interaction consistently with the additive models or did it predict independent behavior consistently. There were a large number of data sets examined, a few of which will be presented herein. The data sets will be divided into two categories Air Force Toxins and Non Air Force Toxins.

Air Force Toxins

The models were fit to a number of data sets, the first data set examined studied malathion and percent protein on percent aniline hydroxylase activity by Bulusu and Chakravarty (1988), #54e2. The data is shown in table 5 and the model parameters are shown in table 24. The independence model provided the most statistically significant fit to the data with a p value of 0.818, the weibull model provided a better fit than the logistic. This indicates that the components are not interacting at the concentrations tested.

Table 24: Independence Model Parameters for Bulusu and Chakravarty (1988), #54e2
Malathion and Percent Protein
Converted to Percent Non Protein
Testing Aniline Hydroxylase Activity

Model Parameters	Copula Logistic	Independence Logistic	Copula Weibull	Independence Weibull
ao	6544.4	54410.82	2.4472	474.37
a1 Compound 1	2.8383e-3	42.192	2.9255e-15	0.4802
a2 Compound 1	3.1621	3.1612	7.2457	0.5512
a1 Compound 2	0.1738	12.4066	8.0161e-2	8.4496e-2
a2 Compound	0.3427	0.3558	0.3164	30.0391
Alpha	1.4052	N/A	2.9313e-3	N/A
Likelihood	6.2378	6.2462	5.2435	5.9694
p - value	0.7159	0.7942	0.8126	0.8178
p - additions	0.9268	N/A	0.3942	N/A

The second data set examined studied the effects of the insecticides aldrin and dieldrin on house fly mortality, by Sun and Johnson (1960), #283e1. The data was shown previously in table 7 and the model parameters are shown below in table 25. The independence models did not fit the data, indicating that the models are not effective to describe binary toxic data.

Table 25: Model Parameters of Sun, Y.P. and Johnson E.R. (1960), #283e1
Aldrin and Dieldrin
House Fly Mortality
Converted to Percent Non Mortality

Model Parameters	Copula Logistic	Independence Logistic	Copula Weibull	Independence Weibull
ao	89.905	118.20	99.270	134.01
a1 Compound 1	48556.4	9812.9	597.49	139.39
a2 Compound 1	2.3966	1.9169	1.4798	1.0745
a1 Compound 2	1924.3	1501.9	195.50	111.62
a2 Compound	1.3705	1.2445	1.0282	0.8697
Alpha	-3.3556	N/A	-3.1494	N/A
Likelihood	9.4121	10.453	4.8694	5.7808
p - value	0.1516	0.1643	0.5606	0.5655
p - additions	0.3075	N/A	0.3397	N/A

The last paper examined studied the acute and chronic effects of endrin and malathion on flagfish mortality. The chronic experiment tested the effects of the pesticides over a 30 day period. The data is shown in table 9 and the model parameters are shown in table 26. The models did not significantly fit the data, indicating that the models are not effective in describing binary toxicity data.

Table 26: Independence Model Parameters for Hermanutz et al. (1985), #121e11
30 Day Mortality of Adult Flagfish
Endrin and Malathion

Parameters	Copula Logistic	Independence Logistic	Copula Weibull	Independence Weibull
a0	4.3553e-15	2.4957e-12	8.7314e-17	1.2538e-70
a1 Cmpd 1	412.69	492.12	56.005	66.442
a2 Cmpd 1	5.2166	5.2608	3.8752	3.9507
a1 Cmpd 2	3.2478e-4	1.3307e-4	2.3242e-4	1.8116e-4
a2 Cmpd 2	2.1590	2.4585	2.2227	2.3152
Alpha	-159.10	N/A	-191.67	N/A
Likelihood	24.550	32.496	18.611	25.603
p - value	4.1345e-4	3.2844e-5	4.8717e-3	5.9289e-4
p - add	4.8185e-3	N/A	8.1918e-3	N/A

The final data set examined from the paper by Hermanutz et al. (1985) tested acute toxicity of endrin and malathion on juvenile flagfish, #121e13. The data is shown in table 12 and the model parameters are shown in table 27. None of the models provided a statistically significant fit to the data, confirming the models poor description to the data.

Table 27: Independence Model Parameters for Hermanutz et al. (1985), #121e13
48 Hour Mortality of Juvenile Flagfish
Endrin and Malathion

Parameters	Copula Logistic	Independence Logistic	Copula Weibull	Independence Weibull
a0	4.2913e-21	3.1334e-9	4.3654e-14	8.3616e-3
a1 Cmpd 1	1.3226e-2	9.6609e-3	1.3003e-2	2.030e-7
a2 Cmpd 1	2.7184e-15	8.9579e-9	2.9384e-9	6.4959e-31
a1 Cmpd 2	9.6264e-7	7.7817e-7	2.4867e-4	2.3392e-4
a2 Cmpd 2	2.5546	2.5971	1.4713	1.4847
Alpha	-71.121	N/A	-112.74	N/A
Likelihood	22.351	23.766	21.217	23.136
p - value	1.0458e-3	1.2524e-3	1.6773e-3	1.6138e-3
p - add	0.2342	N/A	0.1659	N/A

The last data set examined as part of the Air Force toxins studied cleft palate formation in mice litters due to exposure to TCDD and TCDF, by Weber et al. (1985), #293. The data is shown in table 14 and the model parameters from the fits are shown in table 28. All the independence model provided significant fits to the data, however the Copula models provided a significant improvement over the Independence models. The Weibull models provided a better fit to the data than the logistic model.

Table 28: Independence Model Parameters for Weber et al. (1985)
TCDD and TCDF
Cleft Palate Formation in Mice Litters

Parameters	Copula Logistic	Independence Logistic	Copula Weibull	Independence Weibull
a0	8.1319e-15	1.4589e-8	7.5258e-40	2.5425e-12
a1 Cmpd 1	5.4365e-6	1.1679e-5	1.1494e-3	1.7509e-3
a2 Cmpd 1	4.9733	4.7114	2.6386	2.4946
a1 Cmpd 2	1.8245e-10	2.8491e-12	4.9831e-6	4.9471e-7
a2 Cmpd 2	3.8863	4.5812	2.0456	2.4246
Alpha	-203.75	N/A	-301.94	N/A
Likelihood	3.7799	9.9573	3.0481	9.3528
p - value	0.1511	1.8933e-2	0.2178	2.4950e-2
p - add	1.2939e-2	N/A	1.2042e-2	N/A

Non Air Force Toxins

Two data sets will be examined which are not Air Force toxins to show that the independence models can not consistently describe binary toxicity data. The first data set fit by the models tested the effects of two drugs used to dissolve clots in blood vessels by Lu, H.R. et al. (1991), #297e7. The drugs were given by intravenous infusion and the response was changed to percent non clot lysis to meet the limitations of the model. The data is shown in table 18 and the results and model parameters are shown in table 29. All the models provided significant fits to the data, however, p values of the Copula models were greater than 0.05, with the Weibull model providing the best fit.

Table 29: Independence Model Parameters for Lu, H. R. et al. (1991), #294e7 rscu-PA and rt-PA-ΔFE/scu-PA-e by Intravenous Infusion

Model Parameters	Copula Logistic	Independence Logistic	Copula Weibull	Independence Weibull
ao	78.024	83.560	127.53	102.68
a1 Compound 1	3.7374	4.0112	1.9748	1.7661
a2 Compound 1	2.0385	1.8508	0.7834	0.9727
a1 Compound 2	1.0074e6	3.3473e4	71.899	149.53
a2 Compound	3.3560	2.9265	1.1720	1.4656
Alpha	2.0398	N/A	1.6940	N/A
Likelihood	8.1904	10.098	7.8590	8.6747
p - value	0.0848	0.0725	0.0969	0.1228
p - additions	0.1672	N/A	0.3665	N/A

The last data set presented as a Non Air Force toxin tested the effects of two sleep drugs on human patients, by Plummer and Short (1990), #299e1. The data is shown in table 20 and the model parameters are shown in table 30. The Weibull copula provided the best fit to the data but the p value was less than 0.05 and therefore not acceptable. This again indicates that the independence models do not consistently describe binary toxicity data.

Table 30: Independence Model Parameters for Plummer and Short (1990), #299e1 Thiopentone and Midazolam Recorded the Number Asleep

Model Parameters	Copula Logistic	Independence Logistic	Copula Weibull	Independence Weibull
a_o	1.1655e-20	1.1954e-15	5.1052e-18	1.1954e-15
a1 Compound 1	0.2853	0.4149	0.2440	0.3344
a2 Compound 1	1.6810	1.4639	1.3193	1.0859
a1 Compound 2	19.245	26.695	6.8002	8.2618
a2 Compound	1.5653	1.7350	1.2001	1.3117
Alpha	-339.08	N/A	-312.01	N/A
Likelihood	25.321	40.960	25.252	41.322
p - value	0.00137	5.0874e-6	0.0014	4.3709e-6
p - additions	7.6661e-5	N/A	6.1048e-5	N/A

Summary of Independence Models

In summary the independence models fit the data relatively quickly, but did not consistently provide significant fits to the data sets tested. The models were not able to describe independent action or interaction between two components. From the 37 data sets analyzed over the course of the study, a statistically significant fit was obtained for fifteen of them. The copula models showed an improvement in fit over the independence models, indicating interaction in only five cases.

A summary of the data sets presented herein is shown in table 31. The table shows the overall lack of consistent description of binary dose response by the models of independence. The models described only one data set out of the seven presented as interactive. The models do however, indicate the overall improvement in the likelihood using the Weibull equation to describe individual dose response of a single component.

Table 31: Summary of data sets

Experiment Number	Author	P Value	Best Model	Equation Used
#54e2	Bulusu and Chakravarty (1988)	0.8178	Independence	Weibull
#283e1	Sun and Johnson (1960)	no significant fit	None	N/A
#121e11	Hermanutz, et al. (1985)	no significant fit	None	N/A
#121e13	Hermanutz, et al. (1985)	no significant fit	None	N/A
#293	Weber, et al. (1985)	0.2178	Copula	Weibull
#294e7	Lu, et al. (1990)	0.1228	Independence	Weibull
#299e1	Plummer and Short (1990)	no significant fit	None	N/A

Comparison of Additivity and Independence Models

The additive models provided a better description of non interactive and relative interactive behavior of binary toxic mixtures than the independence models. The conclusions of the additive models were different than the conclusions reached using the independence models, when the independence models provided a significant fit to the data. The fits to the data presented in this report is shown in table 32.

The data set of Bulusu and Chakravarty (1988) was described as non interactive by the Independence Weibull model and as synergistic by the Modified Margueles 1 plus model. This discrepancy indicates a significant difference between the conclusions reached by the two approaches. This is also confirmed by the data set of Lu et al. (1990) which also indicated a similar result. The data set presented herein by Plummer and Short (1990) indicates that the independence approach can not be used to predict interactive behavior, since non of the model describe the data, while the additive model, margueles 1 describes interaction. The data sets of Hermanutz et al. (1985) indicate the independence models can not consistently describe non

interactive behavior, since the models do not significantly fit the data. The differences between the two models can not be described since the independence models do not consistently describe the data. Furthermore, the differences between the two models can not be quantified since the independence models do not significantly fit most of the data sets studies.

Table 32: Comparison of Model Fits

Experiment Number	Author	Additive		Independence	
		P Value	Best Model	P Value	Best Model
#54e2	Bulusu and Chakravarty (1988)	0.4132	Modified Margueles 2-logistic	0.8178	W-Independence
#283e1	Sun and Johnson (1960)	0.7606	Ideal-logistic	N/A	None
#121e11	Hermanutz, et al. (1985)	0.7661	Modified Margueles 1 plus-logistic	N/A	None
#121e13	Hermanutz, et al. (1985)	0.2592	Ideal-logistic	N/A	None
#293e1	Weber, et al. (1985)	0.8049	Margueles 1-logistic	0.2178	W-Copula
#294e7	Lu, et al. (1990)	0.3432	Modified Margueles 1-logistic	0.1228	W-Independence
#299e1	Plummer and Short (1990)	0.9590	Margueles 1-logistic	N/A	None

Cumulative List Of Written Publications In Journals

"A New Approach for the Analysis of Mixture Toxicity Data", Water Science and Technology, 26, 9-11, 2345-48 (1992), C.N. Haas.

"A New Quantitative Approach for the Analysis of Binary Toxic Mixtures", Environmental Toxicology and Chemistry, 13:149-156 (1994), C.N. Haas and B.A. Stirling.

(additional publications are currently in preparation)

Student Theses from this Project (all at Drexel University)

Bruce A. Stirling, "A Quantitative Approach For Dose-Response Analysis Of Binary Toxic Mixtures", M.S. (1992).

Sean Kersten, "Analysis Of Binary Toxic Mixtures Using A Model Of Independence", M.S. (1995).

Kaushik Cidambi, "Analysis Of Binary Toxic Mixtures Using A Generalized Additivity Model", M.S. (1995).

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Mr. Sean Kersten - April 1994 - September 1994

INTERACTIONS

Papers presented, etc.

"New Approaches for the Analysis of Mixture Toxicity Data", presented at the 16th Biennial Conference of the International Association on Water Pollution Research and Control, Washington D.C., May 1992.

"Testing for the Presence of Interactive Toxic Effects: A New Quantitative Procedure Based on Isobole Analysis", presented at the Eastern North America Regional (ENAR) Meeting of the Biometric Society/American Statistical Association/ Institute of Mathematical Statistics, Philadelphia, PA, March 1993, with Bruce A. Stirling.

Consultative and advisory functions, etc.

none

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APPENDIX A - SYNOPSIS OF DATA EXAMINED

Study Number: 1
Astrup, A., et.al.
Thermogenic synergism between ephedrine and caffeine in healthy volunteers: a double-blind, placebo-controlled study
Metabolism 40 3 323-329 (1991)

Study Computed? N
Compounds Studied: Ephedrine and Caffeine
Biological Response: Thermogenic and metabolic effect levels in humans

# of Combinations: 8
Dose Levels: [10.0-20.0 mg]/[100-200 mg]
Data Type: Normal

Study Number: 3
Hinks, C.F., Spurr, D.T.
The efficacy and cost benefits of binary mixtures of deltamethrin combined with other insecticides or synergists against grasshoppers at two temperatures
Journal of Agricultural Entomology 8 1 29-39 (1991)

Study Computed? N
Compounds Studied: Deltamethrin, Malathion, Carbaryl, Diazinon, Chlorpyrifos (8 total)
Biological Response: Mortality of grasshoppers at limiting temperatures

# of Combinations: 7
Dose Levels: [5.0 g/ha]/[3.13-50.0 g/ha]
Data Type: Normal

Study Number: 4
Khattak, R.A., and Page, A.L., et al.
Accumulation and Interactions of Arsenic, Selenium, Molybdenum and Phosphorus in Alfalfa
Journal of Enviromental Quality 20 165-168 (1991)

Study Computed? N
Compounds Studied: Arsenic, Selenium, Molybdenum, Phosphate
Biological Response: Alfalfa shoot concentrations measured after growth/uptake

# of Combinations: 12
Dose Levels: [0.05-0.1]x[1.0-4.0]x[0-0.1] mg/L
Data Type: Normal

Study Number: 5
Herkovits, J., and Perez-Coll, C.S.
Synergism and Antagonism Induced by Three Carrier Solvents with t -Retinoic Acid and 6-Aminonicotinamide Using FETAX
Environmental Pollution 69 217-221 (1991)

Study Computed? N
Compounds Studied: Lead and Zinc in solution
Biological Response: (Bufo arenarum) Amphibian Larvae mortality (%)

# of Combinations: 13
Dose Levels: [0-16 mg/L]/[0-32 mg/l]
Data Type: Normal

Study Number: 6
Rayburn, J.R., et.al.
Synergism and Antagonism Induced by Three Carirer Solvents with t-Retinoic Acid and 6-Aminonicotinamide Using FETAX
Bulletin of Environmental Contamination and Toxicology 46 625-632 (1991)

Study Computed? Y
Compounds Studied: DMSO, Acetone, Triethylene Glycol
Biological Response: (Xenopus) frog embryo mortality (# dead) using FETAX

# of Combinations: 9
Dose Levels: Multiple dose over 6 seperate experiments
Data Type: Binomial

Study Number: 14
Schrenk, D., et.al.
Assessment of biological activities of mixtures of polychlorinated dibenzo-(rho)-dioxins: comparison between polychlorinated dibenzo-(rho)-dioxins: comparison between defined mixtures and their constituents
Archives of Toxicology 65 114-118 (1991)

Study Computed? N
Compounds Studied: Polychlorinated dibenzo-p-dioxin mixtures
Biological Response: Inhibition effects on rat hepatocyte and hepatoma cells

# of Combinations:
Dose Levels: Multiple dosages of mixtures given
Data Type: Normal

Study Number: 29
Vezina, M., et.al.
Potentiation of chloroform-induced hepatotoxicity by methyl isobutyl ketone and two metabolites
Canadian Journal of Physiology and Pharmacology 68 1055-1061 (1990)

Study Computed? N
Compounds Studied: Chloroform, Methyl Isobutyl Ketone and two major metabolites
Biological Response: Hepatotoxicity potentiation of chloroform in rats

# of Combinations: 15
Dose Levels: [0.5 mL/kg]/[3.75-7.50 mmol/kg]
Data Type: Normal

Study Number: 31
Gupta, S.L.
Interactive effects of nitrogen and copper on growth of cyanobacterium Microcystis
Bulletin of Environmental Contamination and Toxicology 42 270-275 (1989)

Study Computed? N
Compounds Studied: Copper and Nitrogen Compounds
Biological Response: Cyanobacterium cell cultures, specific growth rate (k)

# of Combinations: 10
Dose Levels: [0-0.5 uM]/[1.0-10.0 mM]
Data Type: Normal

Study Number: 34
Khattak, R.A., et al.
Influence of binary interactions of arsenate, molybdate, and selenate on yield and composition of alfalfa
Journal of Environmental Quality 18 355-360 (1989)

Study Computed? N
Compounds Studied: Arsenate, Molybdenum, and Selenium
Biological Response: Alfalfa root and shoot yields and concentrations

# of Combinations: 16
Dose Levels: [0-1.0 mg/L]/[0.01-5.0 mg/L]/[0-1.0 mg/L]
Data Type: Normal

Study Number: 35
Gruden, N., and Matausic, S.
Some factors influencing cadmium-manganese interaction in adult rats
Bulletin of Environmental Contamination and Toxicology 43 101-106 (1989)

Study Computed? N
Compounds Studied: Cadmium and Manganese
Biological Response: Duodenal transfer and intestinal retention of Mn in rats

# of Combinations: 20
Dose Levels: [0.0-2.0 mg/d/rat]/[0.64-4.28 mg/ml milk]
Data Type: Normal

Study Number: 39
Dikshith, T.S.S., et.al.
Interaction of hexachlorocyclohexane (HCH) and chlorpropham (CIPC) in male rats
Toxicology Letters 45 281-288 (1989)

Study Computed? N
Compounds Studied: Hexachlorocyclohexane (HCH), Chlorpropham (CIPC)
Biological Response: Metabolic and biochemical effects of combinations in rats

# of Combinations: 3
Dose Levels: [60.0 mg/kg/d]/[50.0 mg/kg/d]
Data Type: Normal

Study Number: 41
Umbriet T.H.
Alteration of the acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) by estradiol and tamoxifen
Toxicology 59 163-169 (1989)

Study Computed? Y
Compounds Studied: TCDD, Estradiol, and Tamoxifen
Biological Response: Uterine suppression and acute lethality in mice

# of Combinations: 5
Dose Levels: [0-66 ug/kg/day]/[40 ug/kg]/[1 mg/kd/day]
Data Type: Binary

Study Number: 45
Brondeau, M.T., et.al.
Acetone compared to other ketones in modifying the hepatotoxicity of inhaled 1,2-dichlorobenzene in rats and mice
Toxicology Letters 49 69-78 (1989)

Study Computed? N
Compounds Studied: Dichlorobenzene, Acetone, Ketones, Cyclohexanone
Biological Response: Effects on liver P-450, serum GST, GDLH activity in mice

# of Combinations: 8
Dose Levels: Multiple doseages from [733-14790 ppm]
Data Type: Normal

Study Number: 48
Davis, D., Safe, S.
Dose-response immunotoxicities of commercial polychlorinated biphenyls (PCBs) and their interaction with 2,3,7,8-tetrachlorodibenzo-p-dioxin
Toxicology Letters 48 35-43 (1989)

Study Computed? N
Compounds Studied: Tetrachlorodibenzo-p-dioxin, Polychlorinated Biphenyl mixtures
Biological Response: Immunotoxic response using sheep blood cell bioassay

# of Combinations: 8
Dose Levels: [3.7 nmol/kg]/[5-50 mg/kg]
Data Type: Normal

Study Number: 49
Freundt, K.J., et.al.
Decrease of inhaled toluene, ethyl benzene, m-xylene, or mesitylene in rat blood after combined exposure to ethyl acetate
Bulletin of Environmental Contamination and Toxicology 42 495-498 (1989)

Study Computed? N
Compounds Studied: Toluene, Ethyl Benzene, m-Xylene, Mesitylene, Ethyl Acetate
Biological Response: Blood concentrations in rats after combined exposures

# of Combinations: 48
Dose Levels: [100-720 ppm]/[0-4000 ppm]
Data Type: Normal

Study Number: 51
Harrison, P.T.C., Heath, J.C.
Apparent synergy between chrysotile asbestos and N-nitrosoheptamethyleneimine in the induction of pulmonary tumours in rats
Carcinogenesis 9 12 2165-2171 (1988)

Study Computed? N
Compounds Studied: Chrysotile Asbestos, Metalic Cadmium, N-nitrosoheptamethyleneimine
Biological Response: Induction of pulmonary tumors in rats

# of Combinations: 4
Dose Levels: [0-2.0 mg]/[1.0 mg/wk]/[0.18 mg]
Data Type: Binomial

Study Number: 52
Stratton, G.W., and Smith, T.M.
Interaction of organic solvents with the green alga Chlorella pyrenoidosa
Bulletin of Environmental Contamination and Toxicology 40 736-742 (1988)

Study Computed? Y
Compounds Studied: Ethanol, Acetone, and Atrazine
Biological Response: Green Algae (Chlorella pyrenoidosa), (%) inhibition

# of Combinations: 30
Dose Levels: [0.1-5.0 %v/v]/0.05-0.3 ppm]
Data Type: Normal

Study Number: 54
Bulusu, S., and Chakravarty, I.
Profile of drug metabolizing enzymes in rats treated with paration, malathion, and phosalone under various conditions of protein energy malnutrition
Bulletin of Environmental Contamination and Toxicolgy 40 11-118 (1988)

Study Computed? Y
Compounds Studied: Parathion, Malathion, and Phosalone with Malnutrition
Biological Response: Enzyme activity in 5 groups of rats fed low protein diets

# of Combinations: 15
Dose Levels: [0-200 ug/kg body wt.] for each chemical
Data Type: Normal

Study Number: 61
Szepvolgyi, J., et.al.
Examination of the interaction of decis and dithane in rats
Toxicology 53 107-111 (1988)

Study Computed? N
Compounds Studied: Pyrethroid and Dithiocarbamate
Biological Response: Serum and bowel biochemical activities measured in rats

# of Combinations: 10
Dose Levels: [2.5-10.0 mg/kg]/[12.5-2500 mg/kg] b.m.
Data Type: Normal

Study Number: 64
Simmons, J.E., et.al.
Lethality and Hepatotoxicity of Complex Waste Mixtures
Environmental Research 46 74-85 (1988)

Study Computed? N
Compounds Studied: Complex Waste Combinations: Naphthalene, Phenol, Benzene....
Biological Response: Male rats evaluated for mortality after 24 hr. period

# of Combinations: 0
Dose Levels: Multiple doses with active ingredients
Data Type: Binomial

Study Number: 68
Donnelly, K.C., et.al.
Mutagenic potential of binary mixtures of nitro-polychlorinated dibenzo-p-dioxins and related compounds
Journal of Toxicology and Environmental Health 24 345-356 (1988)

Study Computed? N
Compounds Studied: Nitro-Polychlorinated Dioxins (NMCB, NPCB, NMCDD, NTCDD, BaP...)
Biological Response: Mutagenic potentials using Salmonella/microsome assay

# of Combinations: 80
Dose Levels: [0.15-2.5 ug]/[0.05-5 ug]
Data Type: Normal

Study Number: 69
Khanna, R.N., et.al.
Effect of repeated exposure to lindane and cadmium on lindane metabolism in rats
Toxicology Letters 42 177-183 (1988)

Study Computed? N
Compounds Studied: Lindane and Cadmium
Biological Response: Inhibition of Lindane and heavy metal metabolism in rats

# of Combinations: 4
Dose Levels: [2.0 mg/kg]/[0.2 mg/kg/day] for 35 days
Data Type: Normal

Study Number: 74
Chakraborty, I.C., et.al.
Antagonistic and synergistic effects of lead and selenium in Rattus norvegicus
Toxicology Letters 37 21-26 (1987)

Study Computed? N
Compounds Studied: Lead and Selenium
Biological Response: Chromosomal abnormalities in chronic exposure to rats

# of Combinations: 7
Dose Levels: [0-2.5 mg/100g b.w.]/[0-0.047 mg/100g]
Data Type: Normal

Study Number: 75
Bustamante, C.I., et.al.
Synergism of the Combinations of Imipenem plus Ciprofloxacin and Imipenem plus Amikacin against Pseudomonas aeruginosa and Other Bacterial Pathogens
Antimicrobial Agents and Chemotherapy 31 4 632-634 (1987)

Study Computed? N
Compounds Studied: Imipenem, Ciprofloxacin, Amikacin
Biological Response: Antibiotic resistance of bacterial pathogens

# of Combinations: 8
Dose Levels: [0.0625-32 ug/ml]/[0.0625-128 ug/ml]
Data Type: Normal

Study Number: 76
Mandel, R., and Ryser, H.J.-P.
Mechanism of synergism in the mutagenicity of cadmium and N-methul-N-nitrosourea in Salmonella typhimurium: the effect of pH
Mutation Research 176 1-10 (1987)

Study Computed? N
Compounds Studied: Cadmium and N-methyl-N-Nitrosourea
Biological Response: Toxic effects on Salmonella typhimurium @ varying pH

# of Combinations: 4
Dose Levels: [0-0.5 mM]/[0-160 uM]
Data Type: Normal

Study Number: 80
Nikolaev, V., et.al.
Interaction between glucose diet and ethanol on rat liver microsomal induction and liver plasma membrane damage in chronic hexaclorobenzene intoxication
Archives of Toxicology 60 112-114 (1987)

Study Computed? N
Compounds Studied: Ethanol, Hexachlorobenzene, High/Low Glucose
Biological Response: Induction of liver plasma membrane damage in male rats

# of Combinations: 3
Dose Levels: [0.104 mol/kg][17.5 mmol/kg]/[63 % diet]
Data Type: Normal

Study Number: 81
Haake, J.M., et.al.
Aroclor 1254 as an Antagonist of the Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin
Toxicology Letters 38 299-306 (1987)

Study Computed? N
Compounds Studied: Aroclor, Tetrachlorodibenzo-p-Dioxin (TCDD)
Biological Response: Teratogenic effects in pregnant mice

# of Combinations: 3
Dose Levels: [344-750 umol/kg]/[20 ug/kg]
Data Type: Binary

Study Number: 82
Berger, M.R., et.al.
Combination experiments with very low doses of three genotoxic N-nitrosamines with similar organotropic carcinogenicity in rats
Carcinogenesis 8 11 1635-1643 (1987)

Study Computed? N
Compounds Studied: N-nitrosodiethylamine, N-nitrosopyrrlidine, N-nitrosodiethanolamine
Biological Response: Syncarcinogenic activity of very low doses in male rats

# of Combinations: 13
Dose Levels: Multiple doses administered in mg/kg-day
Data Type: Binomial

Study Number: 95
Nishizumi, M., Masuda, Y.
Enhancing effect of 2,3,4,7,8-pentachlorodibenzofuran and 1,2,3,4,7,8-hexachlorodibenzofuran on diethylnitrosamine hepatocarcinogenesis in rats
Cancer Letters 33 333-339 (1986)

Study Computed? N
Compounds Studied: Pentachlorodibenzofuran, Hexachlorodibenzofuran, Diethylnitrosamine
Biological Response: Hepatocarcinogenesis development in rats

# of Combinations: 3
Dose Levels: [10-100 ug/kg body wt.]/[50 ppm]
Data Type: Binomial

Study Number: 96
Gresele, P., et.al.
Lack of Synergism Between Dazoxiben and Dipyridamole Following Administration to Man
Thrombosis Research 37 231-236 (1986)

Study Computed? N
Compounds Studied: Dazoxiben and Dipyridamole
Biological Response: Metabolic levels in man; Plasma, Platelet, Prostaglandin

# of Combinations: 2
Dose Levels: [200 mg]/[200 mg]
Data Type: Normal

Study Number: 99
Howell, S.R., et.al.
The hepatotoxic potential of combined toluene-chronic ethanol exposure
Archives of Toxicology 59 45-50 (1986)

Study Computed? N
Compounds Studied: Toluene, Ethanol
Biological Response: Hepatotoxic potential tested in male rats

# of Combinations: 3
Dose Levels: [10,000 ppm]/[10.1-11.3 g/kg]
Data Type: Normal

Study Number: 106
Saxena, D.K., et.al.
Embryotoxic and Teratogenic Effects of Interaction of Cadmium and Lindane in Rats
Acta Pharmacology et Toxicology 59 175-178 (1986)

Study Computed? N
Compounds Studied: Lindane and Cadmium
Biological Response: Embryotoxic and tetratogenic effects in rats

# of Combinations: 4
Dose Levels: [20 mg/kg/day]/[100 ppm/day]
Data Type: Normal

Study Number: 113
Arnold, D.L., et.al.
Long-term toxicity of hexachlorobenzene in the rat and the effect of dietary Vitamin A
Food and Chemical Toxicology 23 9 779-793 (1985)

Study Computed? N
Compounds Studied: Hexacholorobenzene, Vitamin A
Biological Response: Long-term toxicity (many parameters tested) in rats

# of Combinations: 4
Dose Levels: [0.0-40.0 ppm]/[0-10x normal levels]
Data Type: Binomial

Study Number: 118
Abou-Donia, B.M., et.al.
The synergism of n-Hexane induced neurotoxicity by methyl isobutyl ketone following subchronic (90 days) inhalation in hens: Induction of hepatic microsomal cytochrome P-450
Toxicology and Applied Pharmacology 81 1-16 (1985)

Study Computed? N
Compounds Studied: Hexane, Methyl Isobutyl Ketone (MiBK)
Biological Response: Induction of hepatic microsomal cytochrome P-450 in hens

# of Combinations: 7
Dose Levels: [0-1000 ppm]/[0-1000 ppm]
Data Type: Normal

Study Number: 121
Hermanutz, R.O., et.al.
Toxicity of endrin and malathion mixtures to flagfish (Jordanella floridae)
Archives of Environmental Contamination and Toxicology 14 307-314 (1985)

Study Computed? Y
Compounds Studied: Endrin and Malathion Mixtures
Biological Response: Mortality of Flagfish over chronic and acute exposures

# of Combinations: 84
Dose Levels: [0.75-0.99 ug/L]/[265-435 ug/L]
Data Type: Normal

Study Number: 124
Doeleman, P., et al.
Synergism and Antagonsim in the Analysis of Inseticide Resistance
Bulletin of Environmental Contamination and Toxicology 32 717-723 (1984)

Study Computed? Y
Compounds Studied: Cadmium and Lead tested over time
Biological Response: Nematode (reproduction) feeding on bacteria in soil

# of Combinations: 9
Dose Levels: [0-12.7 ug/g]/[0-110 ug/g]
Data Type: Normal

Study Number: 130
Dashiell, O.L., Kennedy Jr., G.L.
The Effect of Fasting on the Acute Oral Toxicity of Nine Chemicals in the Rat
Journal of Applied Toxicology 4 6 320-325 (1984)

Study Computed? N
Compounds Studied: 9 total (Adiponitrile, Bromobenzene, Caffeine, Methomyl, Lead...)
Biological Response: Acute toxicity (mortality) using fasted and non-fasted rat

# of Combinations: 91
Dose Levels: Multiple doseage over wide range (mg/kg)
Data Type: Binomial

Study Number: 132
Chakrabarti, S., Brodeur, J.
Influence of Mercuric Chloride on the Metabolism and Hepatoxicity of Bromobenzene in Rats
Environmental Research 39 March 13th 50-59 (1984)

Study Computed? N
Compounds Studied: Mercuric Chloride and Bromobenzene in various combinations
Biological Response: Influence on metabolism and hepatotoxicity in rats

# of Combinations: 4
Dose Levels: [1-2 mg/kg]/[1-2.5 mmole/kg]
Data Type: Normal

Study Number: 136
Stratton, G.W.
Interaction effects of permethrin and atrazine combinations towards several nontarget microorganisms
Bulletin of Environmental Contamination and Toxicology 31 297-303 (1983)

Study Computed? N
Compounds Studied: Permethrin and Atrazine Combinations
Biological Response: Toxicity towards non-target soil microorganisms

# of Combinations: 30
Dose Levels: [0-3.0 ppm]/[0-0.1 ppm]
Data Type: Normal

Study Number: 137
Denda, A., et.al.
Effects of caffeine on pancreatic tumorigenesis by 4-hydroxyamino-quinoline 1-oxide in partially pancreatectomized rats
Carcinogenesis 4 1 17-22 (1983)

Study Computed? N
Compounds Studied: Caffeine and 4-Hydroxyaminoquinoline-1-oxide
Biological Response: Reduction of pancreatic tumorigenesis in rats

# of Combinations: 6
Dose Levels: [0-7.0 mg/kg]/[0-120 mg/kg body wt.]
Data Type: Binary

Study Number: 142
Francis, P.C., and Petersen, R.L.
Synergistic and antagonistic responses of fern spore germination to combinations of copper, cadmium and zinc
Bulletin of Environmental Contamination and Toxicology 30 567-574 (1983)

Study Computed? N
Compounds Studied: Copper, Cadmium, and Zinc
Biological Response: Fern spore germination; 2 species used, (%) mortality

# of Combinations: 15
Dose Levels: [0-10.0 ppm] equal weight ratios used
Data Type: Normal

Study Number: 143
Horvath, P.M., and Ip, C.
Synergistic effect of Vitamin E and selenium in the chemoprevention of mammary carcinogenesis in rats
Cancer Research 43 5335-5341 (1983)

Study Computed? N
Compounds Studied: Vitamin E and Selenium
Biological Response: Chemoprevention of mammary carcinogenesis in female rats

# of Combinations: 4
Dose Levels: [50-1000 mg/kg]/[0.1-2.5 mg/kg]
Data Type: Binomial

Study Number: 153
Zaleska-Freljan, K.I., Kosicka, B.
Influence of Bromfenvinfos Alone and in Mixture with Methoxychlor on the Blood Indices of Laboratory Mice
Polish Journal of Pharmacology and Pharmacy 34 187-192 (1982)

Study Computed? N
Compounds Studied: Bromfenvinfos, and Methoxychlor
Biological Response: Influences on blood indices and weights of laboratory mice

# of Combinations: 3
Dose Levels: [12.23 mg/kg/day]/[24.66 mg/kg/day]
Data Type: Normal

Study Number: 154
Dilley, J.V., et.al.
Short-Term Oral Toxicity of a 2,4,6-Trinitrotoluene and Hexahydro-1,3,5-Trinitro-1,3,5-Triazine Mixture in Mice, Rats, and Dogs
Journal of Toxicology and Environmental Health 9 587-610 (1982)

Study Computed? N
Compounds Studied: Trinitrotoluene, Hexahydro-Trinitro-Triazine (munitions mixture)
Biological Response: Short term oral toxicity in mice, rats, and dogs

# of Combinations: 5
Dose Levels: Multiple doseages of mixture [mg/kg*day]
Data Type: Normal

Study Number: 157
Habs, M., Schmahl, D.
Inhibition of the Hepatocarcinogenic Activity of Diethylnitrosamine (DENA) by Ethanol in Rats
Hepato-gastroenterol 28 242-244 (1981)

Study Computed? N
Compounds Studied: Diethylnitrosamine and Ethanol
Biological Response: Inhibition of hepatocarcinogenic activity in male rats

# of Combinations: 3
Dose Levels: [0.0-0.1 mg/kg]/[0-25% in drinking water]
Data Type: Binomial

Study Number: 158
Clement, L.P.
Factors Influencing the Anticarcinogenic Efficacy of Selenium in Dimethylbenz[a]anthracene-induced Mammary Tumorigenesis in Rats
Cancer Research 41 2683-2686 (1981)

Study Computed? N
Compounds Studied: Selenium and Dimethylbenz[a]anthracene
Biological Response: Tumorigenesis in Rats fed low and high fat diets

# of Combinations: 8
Dose Levels: [0.1-5.0 ppm]/[5-10 mg]; 5-25% fat
Data Type: Binomial

Study Number: 164
Hass, B.S., et.al.
Synergistic, Additive, and Antagonistic Mutagenic Responses to Binary Mixtures of Benzo(a)pyrene and Benzo(e)pyrene as Detected by Strains TA98 and TA100 in the Salmonella/Microsome Assay
Environmental Mutagenesis 3 159-166 (1981)

Study Computed? N
Compounds Studied: Benzo(a)pyrene, Benzo(e)pyrene
Biological Response: Mutagenic response using the Salmonella/Microsome assay

# of Combinations: 24
Dose Levels: [0.25-2.5 ug/plate]/[0.2-2.5 ug/plate]
Data Type: Normal

Study Number: 168
Lamb, J.C., et.al.
Development and Viability of Offspring of Male Mice Treated with Chlorinated Phenoxy Acids and 2,3,7,8-Tetrachlorodibenzo-p-dioxin
Journal of Toxicology and Environmental Health 8 835-844 (1981)

Study Computed? N
Compounds Studied: 2,4,5-T, 2,4-D, TCDD (Chlorinated Phenoxy Acids)
Biological Response: Development and offspring viability inhibition in mice

# of Combinations: 4
Dose Levels: [40-80 mg/kg]/[0.16-2.4 ug/kg]
Data Type: Normal

Study Number: 175
Habs, M., et.al.
Influence of Thioctic Acid (alpha-Lipoic Acid) on N-Nitroso-diethylamine-induced Carcinogenesis in Male Sprague-Dawley Rats
Drug Research 30(II) Nr.10 1715-1717 (1980)

Study Computed? N
Compounds Studied: Thioctic Acid, N-Nitroso-diethylamine
Biological Response: Inhibition of carcinogenesis in male rats

# of Combinations: 3
Dose Levels: [45-180 mg/kg]/[10 mg/kg]
Data Type: Binomial

Study Number: 181
Dajani, E.Z., et.al.
Synergistic Actions of Propantheline Bromide with Cimetidine and Thiopropazate Hydrochloride in the Prevention of Stress Ulcer Formation in Rats
The Journal of Pharmacology and Experimental Therapeutics 210 3 373-377 (1979)

Study Computed? N
Compounds Studied: Bromide, Cimetidine, Thiopropazate
Biological Response: Prevention of stress ulcer formation in rats

# of Combinations: 13
Dose Levels: [1.0-5.6]/[10-300]/[10-300 mg/kg]
Data Type: Binomial

Study Number: 183
Dicks, J.W., Abdel-Kawi, A.A.
Antagonistic and Synergistic Interactions between Ancymidol and Gibberellins in Shoot Growth of Cucumber (Cucumis sativus L.)
Journal of Experimental Botany 30 117 779-793 (1979)

Study Computed? N
Compounds Studied: Ancymidol and Gibberellins
Biological Response: Shoot growth of Cucumber (Cucumis sativus)

# of Combinations: 6
Dose Levels: [0-2.0 mg/dm]/[0-100.0 mg/dm]
Data Type: Normal

Study Number: 202
Berenbaum, M.C.
Synergy, additivism and antagonism in immunosuppression
Clinical and Experimental Immunology 28 1-18 (1977)

Study Computed? N
Compounds Studied: Multiple studies used as references
Biological Response: Dependent upon study.

# of Combinations: N/A
Dose Levels: N/A
Data Type: N/A

Study Number: 203
Kurihara, N., et.al.
Metabolic Detoxication and Synergistic Ration of Lindane Analogs in House Flies
Pesticide Biochemistry and Physiology 7 332-340 (1977)

Study Computed? N
Compounds Studied: Lindane Analogs and Piperonyl Butoxide
Biological Response: Metabolic detoxication using house flies

# of Combinations: 6
Dose Levels: [Varied Dose]/[0-100% per combination]
Data Type: Normal

Study Number: 204
Michel, J., et.al.
Bactericidal Synergistic Effect due to Chloramphenicol Induced Inhibition of Staphyloccal Penicillinase
Chemotherapy 23 32-36 (1977)

Study Computed? N
Compounds Studied: Chloramphenicol and Penicillin-G
Biological Response: Bactericidal effects on resistant Staphyloccus aureus

# of Combinations: 0
Dose Levels: [0-8.0 ug/ml]/[0-12.0 ug/ml]
Data Type: Normal

Study Number: 214
Berenbaum, M.C., et.al.
Synergistic effect of cortisol and prostaglandin E2 on the PHA response: Relation to immunosuppression induced by trauma
Clinical and Experimental Immunology 26 534-541 (1976)

Study Computed? N
Compounds Studied: Cortisol and Prostiglandin E2
Biological Response: PHA response of human peripheral blood lymphocytes

# of Combinations: 30
Dose Levels: Multiple dose at molar (M) concentrations
Data Type: Normal

Study Number: 215
Kennedy Jr., G.L., et.al.
Subacute Toxicity Studies with Sodium Saccharin and Two Hydrolytic Derivatives
Toxicology 6 133-138 (1976)

Study Computed? N
Compounds Studied: Sodium Saccharin, Sulfamoylbenzoic Acid, Ammonium Carboxybenzene
Biological Response: Subacute toxicity studies in dogs and rats

# of Combinations: 14
Dose Levels: [All compounds tested in 0-20000 ppm]
Data Type: Normal

Study Number: 216
Wildman, J.M., et.al.
Benzene and Lead Inhibition of Rabbit Reticulocyte Heme and Protein Synthesis: Evidence for Additive Toxicity of These Two Components of Commercial Gasoline
Research Communications in Chemical Pathology and Pharmacology 13 3 473-488 (1976)

Study Computed? N
Compounds Studied: Benzene and Lead as components in gasoline
Biological Response: Inhibition of rabbit reticulocyte heme/protein synthesis

# of Combinations: 2
Dose Levels: [0.113 M final conc.]/[100 uM]
Data Type: Normal

Study Number: 222
Shinohara, Y., et.al.
Combination effect of citrinin and other chemicals on rat kidney tumorigenesis
Gann *67* 147-155 (1976)

Study Computed? N
Compounds Studied: Citrinin, N-nitrosodimethylamine, N-(dichlorophenyl)succinimide
Biological Response: Combination effect on kidney tumorigenesis in rats

# of Combinations: 8
Dose Levels: [0.02-0.05%]/[0.05%]/[0.05%] in diet
Data Type: Normal

Study Number: 224
Schmahl, D.
Investigations on esophageal carcinogenicity by methyl-phenyl-nitrosamine and ethyl alcohol in rats
Cancer Letters 1 215-218 (1976)

Study Computed? N
Compounds Studied: Methyl-Phenyl-Nitrosamine, Ethyl Alcohol
Biological Response: Esophageal carcinogenicity in rats

# of Combinations: 8
Dose Levels: [58-240 mg/kg]/[30 ml/kg]
Data Type: Binomial

Study Number: 228
Drewinko, B., et.al.
Combination Chemotherapy In Vitro with Adriamycin. Observations of Additive, Antagonistic, and Synergistic Effects When Used in Two-Drug Combinations on Cultured Human Lymphoma Cells
Cancer Biochemistry and Biophysics 1 187-195 (1976)

Study Computed? N
Compounds Studied: Adriamycin in combination with 12 other chemotherapeutic drugs
Biological Response: Lethality and effects on cultured human lymphoma cells

# of Combinations: 6
Dose Levels: [0.25 ug/ml] with multiple doseages
Data Type: Normal

Study Number: 232
Shabad, L.M., et.al.
On the Influence of Chloramphenicol on the Induction of Lung Adenomas by Urethane in Mice
Neoplasma 22 4 347-354 (1975)

Study Computed? N
Compounds Studied: Chloramphenicol and Urethane
Biological Response: Lung adenoma development in different groups of mice

# of Combinations: 4
Dose Levels: [0-4.0 mg/g]/[0-1.0 mg/g]
Data Type: Binomial

Study Number: 241
Gottlieb, S.F., et.al.
Synergistic Action of Increased Oxygen Tensions and PABA-Folic Acid Antagonists on Bacterial Growth
Aerospace Medicine 45 8 829-833 (1974)

Study Computed? N
Compounds Studied: Sodium Sulfisoxazole and Trimethoprim
Biological Response: Effects on bacterial growth @ different oxygen tensions

# of Combinations: 20
Dose Levels: [0-5000 ug%]/[0-100 ug%]
Data Type: Normal

Study Number: 243
Kaufman, D.G., Madison, R.M.
Synergistic Effects of Benzo (a) pyrene and N-Methyl-N-Nitrosourea on Respiratory Carcinogenesis in Syrian Golden Hamsters
Journal of the National Cancer Institute 52 207-218 (1974)

Study Computed? N
Compounds Studied: Methyl-N-Nitrosourea, Benzo(a)pyrene, Ferric Oxide
Biological Response: Respiratory carcinogenesis in Syrian Golden Hamsters

# of Combinations: 4
Dose Levels: [0, 0.5, 5 mg] weekly treatments
Data Type: Binomial

Study Number: 245
Nixon, J.E., et.al.
Effect of Cyclopropenoid Compounds on the Carcinogenic Activity of Diethylnitrosamine and Aflatoxin B1 in Rats
Journal of the National Cancer Institute 53 2 453-458 (1974)

Study Computed? N
Compounds Studied: Cyclopropenoid, Aflatoxin B, Diethylnitrosamine
Biological Response: Effects on carcinogenic activity in rats

# of Combinations: 6
Dose Levels: [0.04-10.0 %]/[20-100 ppb]/[0.2-1 mg/kg]
Data Type: Binomial

Study Number: 246
Cardesa, A., et.al.
Effects of Intraperitoneal Injections of Dimethyl- and Diethlnitrosamine, Alone or Simultaneously on Swiss Mice
Zentral Krebsforschung 82 233-238 (1974)

Study Computed? N
Compounds Studied: Diethylnitrosamine, Dimethylnitrosamine
Biological Response: Rates of tumor formation and incidence in swiss mice

# of Combinations: 4
Dose Levels: [3-6 mg/kg]/[3-6 mg/kg-week] x's 10 weeks
Data Type: Binomial

Study Number: 254
Pound, A.W., et.al.
Increased Carcinogenic Action of Dimethylnitrosamine After Prior Administration of Carbon Tetrachloride
British Journal of Cancer 27 451-459 (1973)

Study Computed? N
Compounds Studied: Dimethylnitrosamine with prior treatment of Carbon Tetrachloride
Biological Response: Potentiation of carcinogenic action in rats

# of Combinations: 4
Dose Levels: [20-40 mg/kg]/[2.5 ml/kg]
Data Type: Binomial

Study Number: 258
Rodriquez, B.P., Lambeth, V.N.
Synergism and Antagonism of GA and Growth Inhibitors on Growth and Sex Expression in Cucumber
Journal of the American Society of Horticultural Science 97 1 90-92 (1972)

Study Computed? N
Compounds Studied: Gibberellic Acid, Maleic Hydrazine, SADH, Ethephon
Biological Response: Inhibition of growth and sex expression in Cucumber

# of Combinations: 3
Dose Levels: Total combinations [100-2000 ppm]
Data Type: Normal

Study Number: 271
Ito, N., et.al.
The Development of Carcinoma in Liver of Rats Treated with m-Toluylenediamine and the Synergistic and Antagonistic Effects with Other Chemicals
Cancer Research 29 1137-1145 (1969)

Study Computed? N
Compounds Studied: m-Toluylenediamine with 3-Methylcholanthrene, m-Toluylenediamine...
Biological Response: Development of carcinoma and liver weight in rats

# of Combinations: 4
Dose Levels: [0.1-0.06]/[0.0067-1.0] % in diet
Data Type: Binomial

Study Number: 277
Deichmann, W.B., et.al.
Synergism among Oral Carcinogens
II. Results of the Simultaneous Feeding of Bladder Carcinogens to Dogs
Toxicology and Applied Pharmacology 7 657-659 (1965)

Study Computed? N
Compounds Studied: 2-Naphthylamine, 4-Nitrobiphenyl
Biological Response: Urinary carcinoma development in female Beagle dogs

# of Combinations: 3
Dose Levels: [0.1 g/dog]/[0.1 g/dog]
Data Type: Binomial

Study Number: 280
Elion, G.B., et.al.
Potentiation by inhibition of drug degradation: 6-substituted purines and xanthine oxidase
Biochemical Pharmacology 12 85-93 (1963)

Study Computed? Y
Compounds Studied: 6 Substituted Purines, Xanthine Oxidase
Biological Response: Inhibition of adenocarcinoma formation in mice

# of Combinations: 48
Dose Levels: Multiple doses (mg/kg body weight)
Data Type: Binomial

Study Number: 281
Bieber, S., et.al.
Suppression of the Immune Response by Drugs in Combination
Proceedings of the Society for Experimental Biology and Medicine 111 334-337 (1962)

Study Computed? Y
Compounds Studied: Thioguanine, Mercaptopurine, Urethan
Biological Response: Suppression of immune system response male mice

# of Combinations: 24
Dose Levels: [0-3.0 mg/kg]/[0-75 mg/kg]/[0-675 mg/kg]
Data Type: Normal

Study Number: 283
Sun, Yun-Pei, and Johnson, E.R.
Analysis of Joint Action Insecticides against House Flies
Journal of Economic Entomology 53 5 887-892 (1960)

Study Computed? N
Compounds Studied: Dieldrin, Aldrin, Lindane, Chlordane, Pyrethrins, and others
Biological Response: Mortality of joint action tested against house flies

# of Combinations: 12
Dose Levels: Multiple doseages in binary combinations
Data Type: Normal

Study Number: 287
Kagy, J.F., and Richardson, C.H.
Ovicidal and Scalicidal Properties of Solutions of Dinitro-o-cyclo-hexylphenol in Petroleum Oil.
Journal of Economic Entomology 29 52-59 (1936)

Study Computed? N
Compounds Studied: Phenol and Petroleum Oil emulsions
Biological Response: Mortality of plant bug eggs measured in net kill (%)

# of Combinations: 18
Dose Levels: [0.0-5.0% in oil]/[1.0-3.0% in spray]
Data Type: Binomial

Study Number: 288
Tattersfield, F., and Martin, J.T.
The Problem of the Evaluation of Rotenone-Containing Plants
Annals of Applied Biology 22 578-605 (1935)

Study Computed? N
Compounds Studied: Rotenone extracted from Derris Root; to be used as reference only
Biological Response: Aphid mortality as an indication of concentration

# of Combinations: 0
Dose Levels: [1.0-30.0%] in solution
Data Type: None

Study Number: 289
Solana. R.P., et.al.
Estimation and Analysis of the Concentration-Response Surfaces Associated with Multiple-Agent Combinations
Toxicology and Applied Pharmacology 85 231-238 (1986)

Study Computed? N
Compounds Studied: Ethylnitrosourea and D-Dichloroplatinum
Biological Response: Sister chromatid exchange activity in chinese hamster cell

# of Combinations: 16
Dose Levels: [0-1000 uM]/[0-10.0 uM]
Data Type: Normal

Study Number: 290
Francis, P.C., and Petersen, R.L.
Effect of Copper, Cadmium, and Zinc on Percent Spore Germination of the Cinnamon Fern (Osmunda cinnamomea) and the Sensitive Fern (Onoclea sensibilis)
Bulletin of Environmental Contamination and Toxicology 30 559-566 (1983)

Study Computed? N
Compounds Studied: Copper, Cadmium, Zinc
Biological Response: Spore germination of Osmunda cinnamomea L. and Onoclea sensibilis L.

# of Combinations: None
Dose Levels: [0-40 ppm]
Data Type: Normal

Study Number: 291
Lidor, Y.J., et al.
Synergistic Cytotoxicity of Different Alkylating Agents for Epithelial Ovarian Cancer
International Journal of Cancer 49 704-710 (1991)

Study Computed? Y
Compounds Studied: Cisplatin, Thiotepa, Melphalan, 4HC, CBDCA,
Biological Response: Ovarian Cancer Cell Lines (OVCA 420, 429, 433; and OVCAR-3)

# of Combinations: Between 8 and 10 combinations per each of 4 cell lines
Dose Levels: [0.08 to 3.8 μM x 0.08 to 7 μM]
Data Type: No response level given.

Study Number: 292
Gallo, M.A., et. al.
Interactive Effects of Estradiol and 2,3,7,8-Tetrachlorodibenzo-p-dioxin on Hepatic Cytochrome P-450 and Mouse Uterus
Toxicology Letters 32 123-132 (1986)

Study Computed? N
Compounds Studied: TCDD and Estradiol
Biological Response: AHH activity, Induction ration, Cytochrome P-450

# of Combinations: 10
Dose Levels: [0 or 72 μg/mouse]x[0-280 ng/mouse]
Data Type: Normal

Study Number: 293
Weber, H.
Teratogenic Potency of TCDD, TCDF and TCDD-TCDF Combinations in C57BL/6N
Toxicology Letters 26 159-167 (1985)

Study Computed? Y
Compounds Studied: TCDD and TCDF
Biological Response: Fetal palates and maternal kidneys

# of Combinations: 8
Dose Levels: [12,17,22];[300,600,900]
Data Type: Binomial and Normal

Study Number: 294
Lu, H.R., et al.
Comparative Thrombolytic Properties of Bolus Injections and Continuous Infusions of a Chimeric (t-PA/u-PA) Plasminogen Activator in a Hamster Pulmonary Embolism Model
Blood 78 125-131 (1991)

Study Computed? Y
Compounds Studied: rt-PA, rscu-PA, rt-PA-ΔFE/scu-PA-e
Biological Response: Hamster pulmonary embolism model

# of Combinations: 22
Dose Levels: [0,0.016, 0.032, 0.064, 0.125, 0.25, 0.5]; [0,0.25,0.5,1,2]; [0,0.004,0.008,0.016,0.032,0.064]
Data Type: Normal

Study Number: 295
Witt, P.A. et al
Norepinephrine and ATP are synergistic in the mouse vas deferens preparation
European Journal of Pharmacology 204 149-155 (1991)

Study Computed? N
Compounds Studied: Norepinephrine and ATP
Biological Response: Mouse Vas Deferens

# of Combinations: 11
Dose Levels: [0.01,0.03,0.1,0.3,1,3,10,100,1000]; [0.03,0.1,1]
Data Type: Normal

Study Number: 296
Nikodijevic, O., et al
Behavioral Effects of A1- and A2- Selective Adenosine Agonists and Antagonists: Evidence for Synergism and Antagonism
The Journal of Pharmacology and Experimental Therapeutics 259 1 286-294 (1991)

Study Computed? N
Compounds Studied: APEC, CHA, NECA
Biological Response: Locomoter Activity in Mice

# of Combinations: 20
Dose Levels: [0,3.7,30]; [0,29,170]; [0,3.2,6.5]
Data Type: Normal

Study Number: 297
Withey, R.J. and J.W. Hall
The Joint Toxic Action of Perchloroethylene with Benzene or Toluene in Rats
Toxicology 4 5-15 (1974)

Study Computed? Y
Compounds Studied: Perchloroethylene, Benzene, Toluene
Biological Response: Rat mortality

# of Combinations: 12
Dose Levels: [0 to 100 by 20]; [0 to 100 by 20]; [0 to 100 by 20]
Data Type: Binomial

Study Number: 298
Williams, C.H. et al
Studies of Toxicity and Enzyme Activity Resulting from Interaction between Chlorinated Hydrocarbon and Carbamate Insecticides
Toxicology and Applied Pharmacology 11 302-307 (1967)

Study Computed? Y
Compounds Studied: Aldrin, Chlordane, Banol, Mobam
Biological Response: Brain, Liver, and Serum Enzymes

# of Combinations: 8
Dose Levels: [70]; [300]; [15.8,31.6]; [45,90]
Data Type: Normal

Study Number: 299
Plummer, J.L. and Short, T.G.
Statistical Modeling of the Effects of Drug Combinations
Journal of Pharmacological Methods 23 297-309 (1990)

Study Computed? Y
Compounds Studied: Rotenone and Pyrethrins
Biological Response: House Flies

# of Combinations: 15
Dose Levels: [0.1 to 0.35 by .05 and .05,.075, 0.1, 0.146, 0.196] x [0 to 1 by 0.25 and 1.5,2,0.375, 0.729, 0.979] mg/mL
Data Type: Binomial

Study Number: 300
McClune, S. et. al
Synergistic Interaction between midazolam and propofol
British Journal of Anaesthesia 69 240-245 (1992)

Study Computed? N
Compounds Studied: Midazolam and Propofol
Biological Response: Patients able to open eyes on command.

# of Combinations: 9
Dose Levels: [0,0.1,0.13,0.16,0.22,0.28,0.34,0.4 and 0.03,0.06,0.12] x [0,0.4,0.8,1.2,1.6,2,2.4,2.8 and 0.3,0.6,0.9]
Data Type: Binomial

Study Number: 302
Finney, D.J.
Probit Analysis
Cambridge University Press 2nd Edition 146-150 (1952)

Study Computed? N
Compounds Studied: Rotenone and Pyrethrins
Biological Response: House Fly mortality

# of Combinations: 10
Dose Levels: [0.25,0.375,0.5,0.729,0.979] X [0.05,0.075,0.1,0.146,0.196] and [0.375,0.5625,0.75,0.125,1.5] X [0.025,0.0375,0.05,0.075,0.1]
Data Type: Binomial

Study Number: 303
Chou, T.C. and Talalay, P.
Analysis of Combined Drug Effects: A New Look at a Very Old Problem
Trends in Pharmacological Science 4 450-454 (1983)

Study Computed? N
Compounds Studied: Rotenone and Pyrethrins
Biological Response: House Fly Mortality

# of Combinations: 10
Dose Levels: [0.25,0.375,0.5,0.729,0.979] X [0.05,0.075,0.1,0.146,0.196] and [0.375,0.5625,0.75,0.125,1.5] X [0.025,0.0375,0.05,0.075,0.1]
Data Type: Binomial

Study Number: 304
Barrai, I. et al
The analysis of the joint effect of substances on reversion systems and the assessment of antimutagenicity
Mutation Research 267 173-182 (1992)

Study Computed? N
Compounds Studied: perylene and cyclopentapyrene
Biological Response: Salmonella typhimurium strain TA98

# of Combinations: 14
Dose Levels: [0.2,0.4,0.6] X [0.25,0.5,1.5,2,3]
Data Type: Normal

Study Number: 306
Zaider, M.
Evidence of a neutron RBE of 70 (+/- 50) for solid-tumor induction at Hiroshima and Nagasaki and its implications for assessing the effective neutron quality factor.
Health Physics 61 5 631-636 (1991)

Study Computed? N
Compounds Studied: N/A
Biological Response: Humans Atomic Bomb Survivors

# of Combinations: N/A
Dose Levels: N/A
Data Type: N/A

APPENDIX B : MATLAB Programs

Matlab Additive Model Binomial Driver

```
%driver for fit - constrained optimization
clear;
global x1 x2 P T Qcum AAcum model cmp1 cmp2 params wfr1  wfr2 transform actparams
p_pred
Qcum=[0.0000];
load #299e3U2;                                  %add name of input data set
Tableau
%**********************SPECIFY      EQUATION      FOR      EACH
COMPONENT************************
cmp1='logistic';
cmp2='logistic';
        %options:
                %  'logistic';
                %  'multistage';
                %  'weibull';

% ****************  SPECIFY MODEL & SET NUMBER OF PARAMETERS
**********************
model= 'modified margueles 1';
        %options:
                %model='ideal';
                %model='margueles 1';
                %model='margueles 1 plus';
                %model='modified margueles 1';
                %model='modified margueles 1 plus';
                %model='margueles 2';
                %model='modified margueles 2';

if strcmp(model,'ideal'),
        params=5;
        actparams=0; end;
%number of activity parameters in overall model
if strcmp(model,'margueles 1'),
        params=6;
        actparams=1; end;
if strcmp(model,'margueles 1 plus'),
        params=7;
        actparams=1; end;
if strcmp(model,'modified margueles 1'),
        params=6;
        actparams=1; end;
if strcmp(model,'modified margueles 1 plus'),
        params=7;
        actparams=2; end;
if strcmp(model,'margueles 2'),
```

```
        params=7;
        actparams=2; end;
if strcmp(model,'modified margueles 2'),
        params=7;
        actparams=2; end;
%
**********************************************************************
**********************
AAcum=zeros(1,params);
x1=Tableau(:,1);
x2=Tableau(:,2);
P=Tableau(:,3);
T=Tableau(:,4);
wfr1=x1 ./ (x1+x2+eps);
%weight fractions  (eps added to prevent zero divide)
wfr2=1-wfr1;
pi=(P+0.1) ./(T+.2);
num_obs=size(pi,1);
        OPTIONS=zeros(1,18);
        OPTIONS(1)=1;                %Display parameter (Default:0). 1 displays some
results
        OPTIONS(2)=1e-4;    %Termination tolerance for X.(Default: 1e-4).
        OPTIONS(3)=1e-4;    %Termination tolerance on F.(Default: 1e-4).
        OPTIONS(4)=1e-7;    %constraint violation;
        OPTIONS(13)=num_obs;    %set equality constraints
        OPTIONS(18)=0.01;  %initial step size
        OPTIONS(14)=150000000; % maximum iterations

guess=[1.893654e-4   1.05120e-00     1.535282e-0   1.0388485e+00    1.3929334e-00-
2.481422];

transform=zeros(params+num_obs,1);
tmp=ones(size(transform(params+1:params+num_obs)));


transform(params+1:params+num_obs)=2*tmp;
    %logist transform for observations;
transform(1)=2;                %logist transform for a0
transform(2:5)=[1 1 1 1];      %log transform for a2 parameters
guess=[guess,pi']';    %combine parameter vector and pi vector
guess=trans(guess,transform);                %transformation of vectors
guess=constr('binomlogistic',guess,OPTIONS);

trguess=invtrans(guess,transform);           %inverse transform
p_pred=trguess((params+1):size(guess,1));

pi=P ./T;
[pi,p_pred]
best=(trguess(1:params))
[f,g]=binomlogistic(guess);
likelihood_t2=f
```

constraints=g

Matlab Binomial Subroutine

```
function [f,g]=binomlogistic(AA);
%binomial likelihood
global T P x1 x2 Qcum AAcum model cmp1 cmp2 params wfr1 wfr2 transform actparams
p_pred
dim=size(T,1);
L=zeros(dim,1);
G=zeros(dim,1);                                  % excess function, zero initially
ending=size(AA,1);
AA=invtrans(AA,transform);                       % inverse transforms
pi=AA(params+1:ending);
a0=AA(1);
pr_obs=P./T;
for i=1:dim,
        if P(i)>0,L(i)=L(i)+P(i)*log(pi(i)/pr_obs(i));end;
        if (T(i)-P(i))>0,L(i)=L(i)+(T(i)-P(i))*log((1-pi(i))/(1-pr_obs(i)));end;
end;
[pr_obs,pi,L];
f=-2*sum(L);                    % return likelihood function;

% CONSTRAINT EVALUATION
dim=size(x1,1);
xs=zeros(dim,1);

if strcmp(cmp1,'logistic'),
        y1=inv_logit(pi,a0,[AA(2);AA(3)]);end;
if strcmp(cmp2,'logistic'),
        y2=inv_logit(pi,a0,[AA(4);AA(5)]);end;
if strcmp(cmp1,'multistage'),
        y1=inv_multistage(pi,a0,[AA(2);AA(3)]);end;
if strcmp(cmp2,'multistage'),
        y2=inv_multistage(pi,a0,[AA(4);AA(5)]);end;
if strcmp(cmp1,'weibull'),
        y1=inv_weibull(pi,a0,[AA(2);AA(3)]);end;
if strcmp(cmp2,'weibull'),
        y2=inv_weibull(pi,a0,[AA(4);AA(5)]);
end;

xs1=x1 ./ (y1+eps);
xs2=x2 ./ (y2+eps);                              %eps added to avoid zero divide
xs=(xs1+xs2); %left hand side of berenbaum equation
g=zeros(dim,1);

%************************  Compute  excess  functions  here
************************************

if strcmp(model,'margueles 1'),
        G=wfr1 .* wfr2 *AA(params);end;
if strcmp(model,'margueles 1 plus'),
        G=wfr1 .* wfr2 .*(AA(params-1) + AA(params) * pi);end;
```

```
if strcmp(model,'modified margueles 1'),
        G=exp(wfr1 .* wfr2 *AA(params)) - 1;end;
if strcmp(model,'modified margueles 1 plus'),
        G=exp((AA(params-1) + AA(params) * pi) .* wfr1 .* wfr2) - 1;end;
if strcmp(model,'margueles 2'),
        G=wfr1 .* wfr2 .* (AA(params-1) + AA(params) * (wfr1 - wfr2));end;
if strcmp(model,'modified margueles 2'),
   G=exp(wfr1 .*wfr2 .*(AA(params-1)+AA(params)* (wfr1 - wfr2))) -1;end

%
**********************************************************************
*******************
for i=1:dim,
        if (x1(i)==0)&(x2(i)==0),xs(i)=pi(i)-a0;      %zero constraint
                else xs(i)=log(xs(i)-G(i));       %replace by log(xs(i)-G)
        end;
end;
g=xs;
AAcum=[AAcum',AA(1:params)]';
Qcum=[Qcum',f]';
W=[Qcum AAcum];
nn=size(W,1);
if rem(nn,10)==0,save scratch W;end;
```

Invtrans Binomial Subroutine

```
function invtransout=invtrans(vectin,transvect);
%performs parameter transformation
% transvect =0  --> no transformation
% transvect =1  --> log transformation
% transvect =2  --> logit transformation
invtransout=vectin;
for i=1:size(vectin,1),
        if transvect(i)==1,invtransout(i)=exp(vectin(i));end;
        if transvect(i)==2,invtransout(i)=1/(1+exp(-vectin(i)));end;
end;
```

Inv_logit Binomial Subroutine

```
function C=inv_logit(pi,a0,A);
%computes concentration given the response pi from logistic model
% with background a0
dim=size(pi,1);
C=zeros(dim,1);
for i=1:dim,
        if (pi(i)>a0)&(pi(i)<1),
                C(i)=((pi(i)-a0)* exp(A(1))/(1-pi(i)))^(1/A(2));
        end;
        if (pi(i)<=a0),C(i)=eps;end;      %tiny value to avoid zero divide
        if (pi(i)==1),C(i)=1e10;end;
end;
```

Inv_multistage Binomial Subroutine

```
function C=inv_multistage(pi,a0,A);
%computes concentration given the response pi from multistage model
% with background a0
dim=size(pi,1);
C=zeros(dim,1);
for i=1:dim,
        if (pi(i)>a0)&(pi(i)<1),
                C(i)=(-A(1)+(A(1)^2 - (4*A(2)*(log(1-pi(i))+a0))))^(1/2))/(2*A(2));
        end;
        if (pi(i)<=a0),C(i)=eps;end;      %tiny value to avoid zero divide
        if (pi(i)==1),C(i)=1e10;end;
end;
```

Inv_weibull Binomial Subroutine

```
function C=inv_weibull(pi,a0,A);
%computes concentration given the response pi from weibull model
```

```
% with background a0
dim=size(pi,1);
C=zeros(dim,1);
for i=1:dim,
        if (pi(i)>a0)&(pi(i)<1),
                C(i)=((-1/A(1))*log((1-pi(i))+a0))^(1/A(2));
        end;
        if (pi(i)<=a0),C(i)=eps;end;      %tiny value to avoid zero divide
        if (pi(i)==1),C(i)=1e10;end;
end;
```

Trans Subroutine

```
function transout=trans(vectin,transvect);
%performs parameter transformation
% transvect =0  --> no transformation
% transvect =1  --> log transformation
% transvect =2  --> logit transformation
transout=vectin;
for i=1:size(vectin,1),
        if transvect(i)==1,transout(i)=log(vectin(i));
        end;
        if transvect(i)==2,transout(i)=log(vectin(i)/(1-vectin(i)));
        end;
end;
```

Matlab Additive Model Normal Driver

```
%driver for fit - constrained optimization
clear;
global x1 x2 Resp StdError Qcum AAcum model cmp1 cmp2 params transform actparams
pred_resp wfr1 wfr2
Qcum=[0.0000];
load #294e7;                          %add name of input data set
Tableau
%*******************SPECIFY EQUATION FOR EACH
COMPONENT***************************
cmp1='logistic';
cmp2='logistic';
        %options:
                %  'logistic';
                %  'multistage';
                %  'weibull';

% ***************  SPECIFY MODEL AND SET NUMBER OF PARAMETERS
**********************
model='modified margueles 1
plus';                                %specify model name
        %options:
                %model='ideal';
                %model='margueles 1';
                %model='margueles 1 plus';
                %model='modified margueles 1';
                %model='modified margueles 1 plus';
                %model='margueles 2';
                %model='modified margueles 2';

if strcmp(model,'ideal'),
        params=5;
        actparams=0; end;                                %number of
activity parameters in overall model
if strcmp(model,'margueles 1'),
        params=6;
        actparams=1; end;
if strcmp(model,'margueles 1 plus'),
        params=7;
        actparams=1; end;
if strcmp(model,'modified margueles 1'),
        params=6;
        actparams=1; end;
if strcmp(model,'modified margueles 1 plus'),
        params=7;
        actparams=2; end;
if strcmp(model,'margueles 2'),
        params=7;
        actparams=2; end;
if strcmp(model,'modified margueles 2'),
```

```
        params=7;
        actparams=2; end;
%
**********************************************************************
******
AAcum=zeros(1,params);
x1=Tableau(:,1);
x2=Tableau(:,2);
Resp=Tableau(:,3);
StdError=Tableau(:,4);
wfr1=x1 ./(x1+x2+eps);
wfr2=1-wfr1;

pi=abs((900-Resp+eps)/1000);

num_obs=size(pi,1);
        OPTIONS=zeros(1,18);
        OPTIONS(1)=1;       %Display parameter (Default:0). 1 displays some results
        OPTIONS(2)=1e-4;    %Termination tolerance for X.(Default: 1e-4).
        OPTIONS(3)=1e-4;    %Termination tolerance on F.(Default: 1e-4).
        OPTIONS(4)=1e-7;    %constraint violation;
        OPTIONS(13)=num_obs;    %set equality constraints
        OPTIONS(18)=0.01;  %initial step size
        OPTIONS(14)=1500000; % maximum iterations
transform=zeros(params+num_obs,1);

guess=[2.936e-3     3.53874e-1   1.516237e-1     1.2614975e-1 2.851505e-1    -2.29e-2
3.5e-2];

tmp=ones(size(transform(params+1:params+num_obs)));
transform(params+1:params+num_obs)=2*tmp;
  %logist transform for observations;
transform(1:5)=[1 1 1 1 1];             %log transform for a0 & a2 parameters
guess=[guess,pi']';             %combine parameter vector and pi vector
guess=trans(guess,transform);  %transformation of vectors
guess=constr('normallogistic',guess,OPTIONS);
trguess=invtrans(guess,transform);

[Resp,pred_resp]
best=(trguess(1:params))
[f,g]=normallogistic(guess);
Obj_func=f
constraints=g
```

Matlab Normal Subroutine

```
function [f,g]=normallogistic(AA);
global StdError Resp x1 x2 Qcum AAcum model cmp1 cmp2 params transform actparams
pred_resp wfr1 wfr2
dim=size(StdError,1);
L=zeros(dim,1);
G=zeros(dim,1);                                          % excess function,
zero initially
ending=size(AA,1);
AA=invtrans(AA,transform);          % inverse transforms
AA=real(AA);
pi=AA(params+1:ending);
contr_resp=AA(1);
pred_resp=(1-pi)*contr_resp;
for i=1:dim,
   if  StdError(i)==0, StdError(i)=eps; end;
        L(i)=((pred_resp(i)-Resp(i))^2)/(StdError(i)^2);
end;
[Resp,pred_resp,L];
f=sum(L);                                                % return likelihood function;
% CONSTRAINT EVALUATION
dim=size(x1,1);
xs=zeros(dim,1);

if strcmp(cmp1,'logistic'),
        y1=inv_logit2(pi,[AA(2);AA(3)]);end;
if strcmp(cmp2,'logistic')
        y2=inv_logit2(pi,[AA(4);AA(5)]);end;
if strcmp(cmp1,'multistage'),
        y1=inv_multistage2(pi,[AA(2);AA(3)]);end;
if strcmp(cmp2,'multistage'),
        y2=inv_multistage2(pi,[AA(4);AA(5)]);end;
if strcmp(cmp1,'weibull'),
        y1=inv_weibull2(pi,[AA(2);AA(3)]);end;
if strcmp(cmp2,'weibull'),
        y2=inv_weibull2(pi,[AA(4);AA(5)]);end;

xs1=x1 ./ (y1+eps);
xs1=real(xs1);
xs2=x2 ./ (y2+eps);                                      %eps added to avoid zero divide
xs2=real(xs2);
xs=(xs1+xs2);                                            %left hand side of berenbaum
equation
g=zeros(dim,1);

%************************* Compute excess functions here
*************************************

if strcmp(model,'margueles 1'),
        G=wfr1 .* wfr2 *AA(params);end;
```

```
if strcmp(model,'margueles 1 plus'),
        G=wfr1 .* wfr2 .*(AA(params-1) + AA(params) * pred_resp);end;
if strcmp(model,'modified margueles 1'),
        G=exp(wfr1 .* wfr2 *AA(params)) - 1;end;
if strcmp(model,'modified margueles 1 plus'),
        G=exp((AA(params-1) + AA(params) * pred_resp) .* wfr1 .* wfr2) - 1;end;
if strcmp(model,'margueles 2'),
        G=wfr1 .* wfr2 .* (AA(params-1) + AA(params) * (wfr1 - wfr2));end;
if strcmp(model,'modified margueles 2'),
   G=exp(wfr1 .*wfr2 .*(AA(params-1)+AA(params)* (wfr1 - wfr2))) -1;end

%
**********************************************************************
******************
for i=1:dim,
        if (x1(i)==0)&(x2(i)==0),xs(i)=pred_resp(i)-Resp(i);  %zero constraint
                else xs(i)=log(xs(i)-G(i));       %replace by log(xs(i)-G)
      end;
end;
g=xs;
g=real(g);
AAcum=[AAcum',AA(1:params)]';
Qcum=[Qcum',f]';
W=[Qcum AAcum];
nn=size(W,1);
if rem(nn,10)==0,save scratch W;
AAcum;
pi;
xs1;
xs2;
y1;
y2;
g;
end;
```

Inv_logit2 Normal Subroutine

```
function C=inv_logit2(pi,A);
%computes concentration given the response Ri from logistic model
% with control response
dim=size(pi,1);
C=zeros(dim,1);
for i=1:dim,
        if (pi(i)>0)&(pi(i)<1),
                C(i)=((1/A(1))*(1/pi(i)-1))^(-1/A(2));end;
        if (pi(i)<=0), C(i)=eps;end;
        if (pi(i)==1), C(i)=1e10;end;
end;
```

Inv_multistage2 Normal Subroutine

```
function C=inv_multistage2(pi,A);
%computes concentration given the response Ri from multistage model
% with control response
dim=size(pi,1);
C=zeros(dim,1);
for i=1:dim,
        if (pi(i)>0)&(pi(i)<1),
                C(i)=(-A(1) + (A(1)^2 - 4*A(2)*(log(1-pi(i))))^(1/2))/(2*A(2));
        end;
        if (pi(i)<=0), C(i)=eps;end;
        if (pi(i)==1), C(i)=1e10;end;
end;
```

Inv_weibull2 Normal Subroutine

```
function C=inv_weibull2(pi,A);
%computes concentration given the response Ri from weibull model
% with control response
dim=size(pi,1);
C=zeros(dim,1);
for i=1:dim,
        if (pi(i)>0)&(pi(i)<1),
                C(i)=((-1/A(1))*(log(1-pi(i))))^(1/A(2));
        end;
        if (pi(i)<=0), C(i)=eps;end;
        if (pi(i)==1), C(i)=1e10;end;
end;
```

MATLAB Independence Models Binomial Driver

```
%fit a binomial copula model
clear;
global x1 x2 P T Qcum AAcum model cmp1 cmp2 transform p_pred
Qcum=[0.0000];
load #121e13;                          %add name of input data set
Tableau
%************SPECIFY EQUATION FOR EACH COMPONENT************
cmp1='logistic';
cmp2='logistic';
        %options;
                %  'logistic'
                %  'weibull'
                %  'multistage'
%************************ SPECIFY MODEL ******************************
model='independence';
        %options;
                        %model='copula';
                        %model='independence';
%*********************************************************************
x1=Tableau(:,1);
x2=Tableau(:,2);
P=Tableau(:,3);
T=Tableau(:,4);
pobs=P./T;
        OPTIONS=zeros(1,18);
        OPTIONS(1)=1;                  %Display parameter (Default:0). 1 displays some results
        OPTIONS(2)=1e-4;    %Termination tolerance for X.(Default: 1e-4).
        OPTIONS(3)=1e-4;    %Termination tolerance on F.(Default: 1e-4).
        OPTIONS(18)=.1;     %initial step size
        OPTIONS(14)=1500000; % maximum iterations

if strcmp(model,'copula'),
        transform=zeros(6,1);
%******************COPULA MODEL - 6 GUESSES*************************
        guess=[5.17350483e-1    5.2170612e-1    7.12394368e-1   3.3745053e-1    1.3051535e-1
-5.33525733e0];
%*********************************************************************
        AAcum=guess;
        guess=[guess]';
        transform(1)=2;
        transform(2:6)=[1 1 1 1 0];
        guess=trans(guess,transform);
end;

if strcmp(model,'independence'),
        transform=zeros(5,1);
%************  INDEPENDENCE MODEL - 5 GUESSES**********************
        guess=[5.17350483e-1    5.2170612e-1    2.12394368e-1   3.3745053e0     1.3051535e-
1];
```

```
%*************************************************************************
        AAcum=guess;
        guess=[guess]';
        transform(1)=2;
        transform(2:5)=[1 1 1 1];
        guess=trans(guess,transform);
end;

guess=fminu('binomlogitcopula',guess,OPTIONS);
trguess=invtrans(guess,transform)
f=binomlogitcopula(guess)
[pobs p_pred]
plot(p_pred,pobs,'yo')
```

MATLAB Independence Models Binomial Subroutine Program

```
function [f]=binomlogitcopula(AA);
%logistic binomial likelihood-copula fit
global T P x1 x2 Qcum AAcum model cmp1 cmp2 params transform p_pred
dim=size(T,1);
L=zeros(dim,1);
AA=invtrans(AA,transform);
AA=real(AA);

a0=AA(1);
pr_obs=P./T;
p1=zeros(dim,1);
p2=zeros(dim,1);
H=zeros(dim,1);
pi=zeros(dim,1);

for i=1:dim,
        if strcmp(cmp1,'logistic'),
                if x1(i) >0,p1(i)=1-logit(x1(i),[AA(2),AA(3)]);else p1(i)=1;end;end;
        if strcmp(cmp2,'logistic'),
                if x2(i) >0,p2(i)=1-logit(x2(i),[AA(4),AA(5)]);else p2(i)=1;end;end;
        if strcmp(cmp1,'weibull'),
                if x1(i) >0,p1(i)=1-weibull(x1(i),[AA(2),AA(3)]);else p1(i)=1;end;end;
        if strcmp(cmp2,'weibull'),
                if x2(i) >0,p2(i)=1-weibull(x2(i),[AA(4),AA(5)]);else p2(i)=1;end;end;
        if strcmp(cmp1,'multistage'),
                if x1(i) >0,p1(i)=1-multistage(x1(i),[AA(2),AA(3)]);else p1(i)=1;end;end;
        if strcmp(cmp2,'multistage'),
                if x2(i) >0,p2(i)=1-multistage(x2(i),[AA(4),AA(5)]);else p2(i)=1;end;end;
end;

if strcmp(model,'copula'),
        H=frank(p1,p2,AA(6));end;
if strcmp(model,'independence'),
        H=frank_ind(p1,p2,0);end;

pi=a0+(1-a0).*(1-H);
p_pred=pi;

for i=1:dim,
        if P(i)>0,L(i)=L(i)+P(i)*log(p_pred(i)/pr_obs(i));end;
        if (T(i)-P(i))>0,L(i)=L(i)+(T(i)-P(i))*log((1-p_pred(i))/(1-pr_obs(i)));end;
end;

[pr_obs,pi,L];
f=-2*sum(L);                                    % return likelihood function;

if strcmp(model,'copula'),
        AAcum=[AAcum',AA(1:6)]';end;
if strcmp(model,'independence'),
```

```
        AAcum=[AAcum',AA(1:5)]';end;

Qcum=[Qcum',f]';
W=[Qcum AAcum];
nn=size(W,1);
if rem(nn,10)==0,save scratch W;end;
```

MATLAB Independence Models Normal Driver Program

```
%fit a normal copula model
clear;
global x1 x2 Resp StdError Qcum AAcum model cmp1 cmp2 transform pred_resp
Qcum=[0.0000];
load #294e13;                          %add name of input data set
Tableau
%************SPECIFY EQUATION FOR EACH COMPONENT************
cmp1='weibull';
cmp2='weibull';
        %options;
                %  'logistic'
                %  'weibull'
                %  'multistage'
%************************ SPECIFY MODEL ****************************
model='copula';
        %options;
                        %model='copula';
                        %model='independence';
%*********************************************************************
x1=Tableau(:,1);
x2=Tableau(:,2);
Resp=Tableau(:,3);
StdError=Tableau(:,4);
        OPTIONS=zeros(1,18);
        OPTIONS(1)=1;              %Display parameter (Default:0). 1 displays some results
        OPTIONS(2)=1e-4;   %Termination tolerance for X.(Default: 1e-4).
        OPTIONS(3)=1e-4;   %Termination tolerance on F.(Default: 1e-4).
        OPTIONS(18)=.1;    %initial step size
        OPTIONS(14)=1500000; % maximum iterations

if strcmp(model,'copula'),
        transform=zeros(6,1);
%******************COPULA MODEL - 6 GUESSES*************************
        guess=[ 2.15347813e-1  4.45267e0  5.0332814530e-1  3.83452917e-1  1.452481746e0
6.35946421e-1 ];
%*********************************************************************
        AAcum=guess;
        transform(1:6)=[1 1 1 1 1 0];                          %log transform for
dose-response parameters
end;

if strcmp(model,'independence'),
        transform=zeros(5,1);
%**************  INDEPENDENCE MODEL - 5 GUESSES**********************
        guess=[ 5.3102164e0    5.108039e-1    2.704127e-1  3.3070136e0   6.1709615e-1];
%*********************************************************************
        AAcum=guess;
        transform(1:5)=[1 1 1 1 1];                                    %log
transform for dose-response parameters
```

```
end;
guess=[guess]';
guess=trans(guess,transform);
guess=fminu('normallogitcopula',guess,OPTIONS);
tr_guess=invtrans(guess,transform)
f=normallogitcopula(guess)
[Resp pred_resp]
plot(pred_resp, Resp,'yo')
```

MATLAB Indepdence Models Normal Subroutine

```
function [f]=normallogitcopula(AA);
%logistic normal likelihood-copula fit
global StdError Resp x1 x2 Qcum AAcum params model cmp1 cmp2 transform pred_resp
dim=size(StdError,1);
L=zeros(dim,1);
AA=invtrans(AA,transform);          % inverse transforms
a0=AA(1);
p1=zeros(dim,1);
p2=zeros(dim,1);
H=zeros(dim,1);
pi=zeros(dim,1);

for i=1:dim,
        if strcmp(cmp1,'logistic'),
                if x1(i) >0,p1(i)=1-logit(x1(i),[AA(2),AA(3)]);else p1(i)=1;end;end;
        if strcmp(cmp2,'logistic'),
                if x2(i) >0,p2(i)=1-logit(x2(i),[AA(4),AA(5)]);else p2(i)=1;end;end;
        if strcmp(cmp1,'weibull'),
                if x1(i) >0,p1(i)=1-weibull(x1(i),[AA(2),AA(3)]);else p1(i)=1;end;end;
        if strcmp(cmp2,'weibull'),
                if x2(i) >0,p2(i)=1-weibull(x2(i),[AA(4),AA(5)]);else p2(i)=1;end;end;
        if strcmp(cmp1,'multistage'),
                if x1(i) >0,p1(i)=1-multistage(x1(i),[AA(2),AA(3)]);else p1(i)=1;end;end;
        if strcmp(cmp2,'multistage'),
                if x2(i) >0,p2(i)=1-multistage(x2(i),[AA(4),AA(5)]);else p2(i)=1;end;end;
end;

if strcmp(model,'copula'),
        H=frank(p1,p2,AA(6));end;
if strcmp(model,'independence'),
        H=frank_ind(p1,p2,0);end;

pi=1-H;

pred_resp=(1-pi)*a0;
for i=1:dim,
        if StdError(i)==0,StdError(i)=eps;end;
        L(i)=((pred_resp(i)-Resp(i))^2)/(StdError(i)^2);
end;
[Resp,pred_resp,L];
f=sum(L);                                   % return likelihood function;

if strcmp(model,'copula'),
        AAcum=[AAcum',AA(1:6)]';end;
if strcmp(model,'independence'),
        AAcum=[AAcum',AA(1:5)]';end;

Qcum=[Qcum',f]';
W=[Qcum AAcum];
```

```
nn=size(W,1);
if rem(nn,10)==0,save scratch W;end;
```

Franks Function Subroutine

```
function H=frank(p1,p2,alpha);
%computes Frank copula;
alpha=real(alpha);
temp=1+(exp(-alpha.*p1)-1).*(exp(-alpha.*p2)-1)./(exp(-alpha)-1);
H=-(1/alpha).*log(temp);
```

Franks Independence Function Subroutine

```
function H=frank_ind(p1,p2,alpha);
% computes Frank_ind copula;
H=p1.*p2;
```

Transform Subroutine

```
function transout=trans(vectin,transvect);
%performs parameter transformation
% transvect =0  --> no transformation
% transvect =1  --> log transformation
% transvect =2  --> logit transformation
transout=vectin;
for i=1:size(vectin,1),
        if transvect(i)==1,transout(i)=log(vectin(i));end;
        if transvect(i)==2,transout(i)=log(vectin(i)/(1-vectin(i)));end;
end;
```

Inverse Transform Subroutine

```
function invtransout=invtrans(vectin,transvect);
%performs parameter transformation
% transvect =0  --> no transformation
% transvect =1  --> log transformation
% transvect =2  --> logit transformation
invtransout=vectin;
for i=1:size(vectin,1),
        if transvect(i)==1,invtransout(i)=exp(vectin(i));end;
        if transvect(i)==2,invtransout(i)=1/(1+exp(-vectin(i)));end;
end;
```

Logistic Subroutine

```
function L=logit(x,a);
%computes logit response, based on 0-1 range
%external background correction required
%a is parameter vector
a0=a(1);a1=a(2);
x=x+(x==0).*eps;      %add epsilon to zero dose
L=1./(1+1./(a0.*x.^a1));
```

Weibull Subroutine

```
function L=logit(x,a);
%computes weibull response, based on 0-1 range
%external background correction required
%a is parameter vector
a0=a(1);a1=a(2);
x=x+(x==0).*eps;      %add epsilon to zero dose
L=1 - exp(-a0.*x.^a1);
```

Multistage (2 stage) Subroutine

```
function L=logit(x,a);
%computes weibull response, based on 0-1 range
%external background correction required
%a is parameter vector
a0=a(1);a1=a(2);
x=x+(x==0).*eps;      %add epsilon to zero dose
L=1 - exp(a0.*x + a1.*x^2);
```